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Fish Welfare

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Acknowledgements

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Thanks is again extended to **Dr. Rose** for permission to reprint his critique addressing the issue of trout and pain sensation. Gratitude is offered to the **American Fisheries Society** (AFS; http://www.fisheries.org) for permission to reprint four of their significant papers and policy statements regarding guidelines for the use of fish in field research. Thanks also to the **Institute for Laboratory Animal Research** (ILAR; http://dels.nas.edu/ilar), a division of The National Academies National Research Council, for permission to reprint important guidelines for the care and use of fish in research. Gratitude is also extended to the **Fisheries Society of the British Isles** (FSBI; http://www.le.ac.uk/biology/fsbi/) for permission to reprint their Briefing Paper 2 which provides a comprehensive look at general fish welfare. Thanks is proffered to **Dr. V. Thorsteinsson** and **Concerted Action "Improvements of Tagging Methods for Stock Assessment and Research in Fisheries" (CATAG)** (http://www.hafro.is/catag/) for permission to reprint their report regarding fish welfare and health in relation to tagging. And last but not least, appreciation is extended to the **Nature Publishing Group** (http://npg.nature.com), **Lab Animal** (http://www.labanimal.com), and **Drs. M. Matthews, B. Trevarrow, and J. Matthews** of the **University of Oregon** and (http://www.uoregon.edu/) **Zebrafish International Research Center** (http://zfin.org/zf_info/stckctr/stckctr.html) for permission to reprint key information for the care and use of zebrafish in research. These international and national selected guidelines and review articles, along with the inclusion of the reprint of the AWIC Bulletin article on the responsible care and health maintenance of fish in commercial aquaculture, present a valuable overview of general fish welfare and topic related fish welfare issues relating to the field, aquaculture, and laboratory research.

The editor also gratefully acknowledges Dr. Donald V. Lightner and Kristie A. Vanpatten of **The University of Arizona Aquaculture Pathology Laboratory** (http://microvet.arizona.edu/research/aquapath/index.htm), for providing the electrophoretic gel picture of polymerase chain reaction products photograph for use on the cover collage. Thanks also to Dr. Bernd Ueberschaer of **LarvalBase** (http://www.larvalbase.org) for granting the permission to use C. Kühn's *Gadus morhura* egg picture on the cover collage. All other photographs found in the cover collage are modified from photos obtained from the USDA-Agricultural Research Service Photo Library.

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in aquatic sciences and fisheries, for the use of 56 abstracts from the ASFA Abstracts database. ASFA is made available from CSA under an agreement between CSA and the Food and Agriculture Organization of the United Nations (FAO), which owns the copyright in ASFA, on behalf of the ASFA Advisory Board. More information on CSA and ASFA is available at http://www.csa.com.

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The Animal Welfare Information Center also gratefully acknowledges the **Institute for Laboratory Animal Research (ILAR)**, an organization whose mission is to foster knowledge about laboratory animal care and use for the scientific community, institutional animal care and use committees (IACUCs), the federal government, science educators and students, and the public. Abstracts are reprinted with permission from ILAR Journal, Institute for Laboratory Animal Research, National Academy of Sciences, 500 Fifth Street, NW, Washington, DC 20001 (http://dels.nas.edu/ilar). To purchase copies of the articles and the entire issue in which it appears, email <ilarj@nas.edu>.

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Preface

Fish are a valuable commodity to the United States, providing: 1) recreation in the form of angling, 2) food and feed products from aquaculture and fisheries, 3) jobs in the aquaculture and fisheries industries, and are 4) excellent alternative lower phyla animals for necessary research. The use of fish in research has been increasing over the last 10 years (DeVita 1984; Post 1987; Powers 1989; Goodrich 1990; Evans 1993; Stoskopf 1993). At the same time, the progress of fish as a food source has also grown, with aquaculture being one of the most rapidly expanding food industries. This in part is due to the fact that fish are seen as a low-fat food source, as a replacement for fat-rich meats, and consequently more emphasis is being place on the amount of fish consumed, the quality of fish produced, and the efficiency of fish growth (DeTolla et al. 1995). Aquaculture research facilitates addressing questions regarding environmental pollution, conservation, and protection of the freshwater estuarine and marine environment (DeTolla et al. 1995). And these factors in turn should also stimulate research performed directly on fish in their natural habitat, where fish are also subjected to environmental stresses (man-made and otherwise) that can harm their health and well-being (DeTolla et al. 1995). Besides aquaculture and fisheries research, fish are increasingly used in the laboratory as animal models in toxocologic, pharmacologic, and genetic studies that might otherwise employ mice or other mammalian species, as a result to greater concerns about the humane use of higher vertebrates in research (DeTolla et al. 1995). A great deal of what we need to know regarding fish welfare is yet to be discovered as the scientific study of fish welfare is at an early stage compared to research efforts on other vertebrates (FSBI 2002). In all, these activities and research increase the knowledge base on the care and use of these species in aquaculture, fisheries, and the laboratory.

People whose sports or hobbies involve fish, whose professional work involves fish, and who are concerned with the general welfare of animals search for the answers to the questions regarding the consequences of human activities on fish welfare (FSBI 2002). As you may or may not be aware of, the topic of fish welfare has been receiving much debate lately in the United States. This subject is being discussed related to angling, aquaculture, general neurophysiology, fisheries, laboratory research, aquariums, and regulation, with the issue of whether or not fish feel pain at the forefront. This increase in public concern is witnessed by numerous web sites, commentaries and reports, not only in the United States, but worldwide as well. And mounting concern for fish welfare is also echoed in the activities of those studying or using fish (FSBI 2002).

Laws, government policies, procedures, and protocols that require humane treatment of animals for all uses (e.g. Animal Welfare Act (7 U.S.C. 2131 et seq.), *Guide for the Care and Use of Laboratory Animals* originally by the National Institutes of Health and revised by the National Research Council (1996), *Policy on the Humane Care and Use of Laboratory Animals* by the Public Health Service (PHS; 1997), and federally mandated "institutional animal care and use committees" (IACUC)) came about due to trepidations regarding the welfare and use of animals in biomedical laboratory research. These research projects are thus reviewed to make sure that projects using animals are necessary and conducted as humanely as possible.

Evaluation of animal well-being should be based on subtle behavioral and physiological changes as well as established environmental limits (FSBI 2002). Because fish are different in ways that are important when considering their welfare, including species, body temperature, stocking densities, vulnerability to poor or polluted water quality, and context dependent characteristics, it can be deceptive to extrapolate from what we know about the welfare of mammals and birds to fish, (FSBI 2002). Therefore, common criteria for welfare of other vertebrate animals should be modified to include fish related criteria, as listed above, before welfare criteria can be usefully applied to fish (FSBI 2002).

The optimum health requirements for major farm-raised species are known. However, requirements for other species are being determined by ongoing research that aims at defining the unique limits of each. Consequently, the amount of information available concerning health requirements varies considerably depending on the species. An understanding of the health requirements for a species increases with the length of time it is commercially cultured and its economic importance. We know much more about how to evaluate the well-being of traditionally cultured species, such as channel catfish, goldfish, fathead minnows, golden shiners, rainbow trout, various ornamentals, and zebrafish than we do about newer culture species.

Respect, for all forms and systems of life, is an intrinsic attribute of scientists and managers who conduct any type of research on fish. The respectful treatment of wild and cultured fishes in research is both an ethical and a scientific necessity (AFS Policy Statements # 16, 22, 30) Traumatized animals (including fish) may show signs of abnormal physiological, behavioral, and ecological responses that defeat the purposes of the investigation (AFS Policy Statements # 16, 22, 30). Because of the very considerable range of adaptive diversity and husbandry requirements represented by the over 20,000 species of fishes, no concise or specific compendium of approved methods and guidelines for fish research is practical or desirable (AFS Policy Statements # 16, 22, 30; DeTolla et al. 1995). The attainment of new knowledge and understanding comprises a major justification for any investigation, with the definitive responsibility for the ethical and scientific validity of an investigation and the methods employed resting with the investigator (AFS Policy Statements # 16, 22, 30; DeTolla et al. 1995).

Presently, the Animal Welfare Act does not cover cold-blooded vertebrates. In response to the 1985 amendment to the Animal Welfare Act, that extended principles of humane laboratory animal care to field research activities, the American Fisheries Society – in cooperation with the American Society of Ichthyologists and Herpetologists, and the American Institute of Fishery Research Biologists – developed and published the *"Guidelines for Use of Fishes in Field Research"* (Nickum 1988) to build on and extend these ethical guidelines to the field, thus promoting the conduct of all fisheries work in a humane manner that eliminates cruelty and minimizes suffering (AFS Policy Statement # 22).

In PHS funded institutions, the *Guide* covers all vertebrate species, but the specific use of fish and other cold blooded species is not addressed. Even though the *Guide* and PHS *Policy* do not provide guidelines for the use of laboratory fish, the Office of Laboratory Animal Welfare (OLAW) states, 'Many of the principles embodied in the *Guide,* although not specifically addressing cold-blooded vertebrates, generally can be adapted to animal care and use programs for various kinds of amphibians, reptiles, and fishes (1996)' (Matthews et al. 2002). All institutions are expected to care for and use fish in research in a manner judged to be professionally and humanely appropriate for the particular species in question (DeTolla et al. 1995). Although fish differ from both warm-blooded and other cold-blooded species, like their endothermic counterparts they need to be maintained in a controlled environment with a limitation on stress (DeTolla et al. 1995).

To assist persons using fish in all types of research, numerous reviews and guidelines have been prepared by various experts in each of the fields. The American Fisheries Society has put out several policy statements regarding the use of fish in general and in field research. Thorsteinsson (2002) published a report on fish welfare and health related to tagging methods for fish research. Schwedler and Johnson (2000) produced an article discussing the responsible husbandry of fish in commercial aquaculture. DeTolla et al. (1995) published guidelines for the care and use of fish in research. Westerfield (2000) published the guide for the laboratory use of zebrafish, which was followed up by Matthews et al. (2002) who published a review of the *Guide* for zebrafish care and users. As a general look at the current state of fish welfare, FSBI (2002) produced a report addressing general fish welfare. The Canadian Council on Animal Care (CCAC) has posted its second draft of CCAC guidelines on: the care and use of fish in research, testing, and teaching. The

draft guidelines and future final guidelines are available at the CCAC webpage (http://www.ccac.ca and http://www.ccac.ca/english/new/newframe.htm).

As the concerns about fish welfare are heating up and not all information can be easily obtained in one source. The Information Resources on Fish Welfare has been designed to provide the most current worldwide data available regarding fish welfare for use by both those who have knowledge in one of the various fish related fields or may even be professionals in a fish related field, as well as for individuals who are interested in learning more about fish welfare issues. This publication does not present an opinion on the subject but is rather a comprehensive review of the available information resources regarding fish welfare and its related issues. In this timely publication, AWIC, in cooperation with various authors and publishing houses, provides ten national and international current review articles and guidelines, which cover the topics of general fish welfare, pain and awareness related to fish, and fish welfare related to aquaculture, laboratory and field research, and fisheries. In addition to the review articles, a thorough review of the literature (including citations with abstracts and web sites) is presented, including the following topics: 1) general fish welfare related topics: alternatives; anesthesia and euthanasia; awareness, cognitive ability, and fear; pain and distress; and health and welfare; 2) culture, fisheries, and research related topics: angling; aquaculture; fisheries; laboratory; aquarium fishes (including general topics, ornamentals, dealers, and pet shops); and selected husbandry topics (including animal domestication, harvest and slaughter, holding and transport, and tagging); and 3) regulatory issues (including a table of national and international animal welfare acts related to fish). As an additional resource for institutional animal care and use committees (IACUC), a section on fish related IACUC web resources are provided. Educational training materials and courses are presented for those wishing to delve further into educating themselves and their facility employees about fish welfare.

AWIC presents this material to provide the various fish communities and regulatory agencies worldwide a comprehensive resource on fish welfare. As this publication does not present an opinion regarding fish welfare, we at USDA AWIC hope that the national and international readers (producers, researchers, IACUC members, government representatives, aquarists, and general public) will use the scientifically based guidelines and information to answer the questions regarding the impact of human activities on fish welfare in his or her field, for his or her species, and to act accordingly to prepare and follow humane procedures for the care and husbandry of aquatic animals. This publication is also presented as a resource for US Federal Government grant applicants and awardees that will use fish in their proposed research.

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How to Use This Document

This publication is a comprehensive review of the available information resources regarding fish welfare and its related issues. Bibliography citations are arranged alphabetically according to the last name of the primary author. Each entry also contains abstracts, if available, descriptors, and the NAL call number if the record was retrieved from the National Agricultural Library's Agricola database. If the full-text of the article/resource is available on the WWW, the URL is provided. At the end of each subsection are listings of World Wide Web sites that will provide additional information on the topic. Readers are cautioned as to the dynamic nature of the internet and the fact that addresses and content are subject to change. Web addresses are current as of July 2003.

This document is divided into seven sections: 1) introduction, 2) selected key review articles, 3) a comprehensive review of the literature available in electronic databases, 4) fish related institutional animal care and use committee (IACUC) web resources, 5) current educational training materials and courses, 6) Cooperative State Research, Education, and Extension Service (CSREES) Current Research Information System Reports (CRIS), and 7) additional records from the National Agricultural Library Electronic Catalog.

This document also contains two appendicies: 1) a list of aquaculture and fisheries professional societies and groups and their web addresses and 2) National Agricultural Library Document Delivery Services instructions for USDA employees and the public.

Selected Review Articles

Twelve key review articles written by national and international experts in various topics relating to fish welfare are included in this section. Each article concludes with a list of references cited by the author.

Review of the Literature

The selected records in this section were compiled from multiple sources and numerous databases including, but not limited to Agricola, Medline, NTIS, AGRIS, CAB International, BIOSIS, and ASFA databases. Citations listed in this section may or may not overlap with articles cited by the introductory authors. Each section also has relevant websites that will provide additional material not found in journals or databases. World Wide Web addresses are listed to access specialized databases, extension materials, and publications produced by a variety of non-profit organizations.

Fish Related Institutional Animal Care and Use Committee (IACUC) Web Resources

The selected relevant materials regarding fish welfare in research and the IACUC in this section were compiled from the internet. The URLs are listed to access specialized databases and linkages, extension materials, and publications produced by a variety of non-profit organizations.

Educational Training Materials and Courses

The selected relevant educational training materials and courses in this section were compiled from multiple sources and the internet. Those materials cited also have relevant websites listed that will provide additional information regarding the educational training materials or courses.

Cooperative State Research, Education, and Extension Service (CSREES) Current Research Information System (CRIS) Reports

Records in this section were retrieved from the Current Research Information System maintained by the Cooperative State Research, Education, and Extension Service. CRIS is the U.S. Department of Agriculture's (USDA) documentation and reporting system for ongoing and recently completed research projects in agriculture, food and nutrition, and forestry. Projects are conducted or sponsored by USDA research agencies, state agricultural experiment stations, the state land-grant university system, other cooperating state institutions, and participants in a number of USDA research grant programs. It is available on the web at http://cris.csrees.usda.gov/.

National Agricultural Library Electronic Catalog

Generally, this resource is closely related to Agricola. However, some relevant materials not appearing in the Agricola database were retrieved and included here.

Aquaculture and Fisheries Professional Societies and Groups

This section contains a list of topic related professional societies and groups available from electronic sources. The URL is listed below each citation and should be accessed to obtain further contact information for the individual professional society or group.

National Agricultural Library Document Delivery Information

The information contained here provides directions on how to obtain copies of articles mentioned in the bibliography from the National Agricultural Library.

1. INTRODUCTION

Introduction

The issue of fish welfare has become increasingly important in the United States and other countries. The impact of human activities on fish welfare are not just in biomedical and other laboratory research, but also in aquaculture, fisheries, angling, and public display.

The production of a fish welfare information resource at this moment in history is particularly appropriate, since fish welfare issues are being debated worldwide and are frequently discussed by scientists not working in fish related fields, by the media, and by the press. This subject is being discussed related to angling, aquaculture, general neurophysiology, fisheries, biomedical laboratory research, aquariums, and regulation.

In accordance with the United States Animal Welfare Act, the use of alternative lower phyla animals, including fish, in research that would otherwise use mammalian species, is encouraged. Numerous species of fish are increasingly being used as biomedical models in comparative genomics, gene expression, transgenesis, carcinogenesis, toxicology, pharmacology, infectious disease, neurology, and aging research, as well as in aquaculture and fisheries related research. And in turn, all of the research conducted in these areas increase the knowledge base on the care and use of these fish species in aquaculture, fisheries, and the laboratory.

To date, fish and other cold-blooded animals are not covered by the Animal Welfare Act. But, different funding agencies, such as USDA, the National Institutes of Heath, and the Public Health Service require adherence to additional guides and policies.

All of the different rules and guidelines can be confusing to anyone conducting research with fish or on fish. But, because of the very considerable range of adaptive diversity and husbandry requirements represented by the over 20,000 species of fishes, no concise or specific compendium of approved methods and guidelines for fish research is practical or desirable (AFS Policy Statements #16, DeTolla et al. 1995).

The Information Resources on Fish Welfare should assist the many scientists, IACUC members, producers, administrators, legislators, and regulatory agencies concerned with general fish welfare and well-being. This book also provides relevant information to those in the general public who benefit from fish related biomedical research, who consume aquaculture or fisheries products, engage in recreational fisheries, keep aquariums or visit aquatic animal displays, as well as those belonging to organizations concerned with animal welfare.

The Information Resources on Fish Welfare will thus serve as an essential comprehensive resource and reference book for a very broad audience, and its Editor is to be congratulated on undertaking the task of producing this unique document. All who read this book should find it useful to guide them in preparing and following appropriate procedures for the care and use of fish.

Meryl C. Broussard, Chair
Joint Subcommittee on Aquaculture
Cooperative State Research, Education, and Extension Service
United States Department of Agriculture

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AFS Policy Statement #16. American Fisheries Society (AFS), American Society of Ichthyologists and Herpetologists (ASIH), and American Institute of Fishery Research Biologists (AIFRE) (1987) Guidelines for the Use of Fishes in Field Research. Approved Sept 1987, Winston-Salem, NC. Published Mar-Apr 1988, Fisheries 13(2):16-23.
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http://dels.nas.edu/ilar/jour_online.asp?id=jour_online

2. SELECTED REVIEW ARTICLES

THE ROYAL SOCIETY

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2.0.

Do fishes have nociceptors? Evidence for the evolution of a vertebrate sensory system

Lynne U. Sneddon[1*], Victoria A. Braithwaite[2] and Michael J. Gentle[1]

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Nociception is the detection of a noxious tissue-damaging stimulus and is sometimes accompanied by a reflex response such as withdrawal. Pain perception, as distinct from nociception, has been demonstrated in birds and mammals but has not been systematically studied in lower vertebrates. We assessed whether a fish possessed cutaneous nociceptors capable of detecting noxious stimuli and whether its behaviour was sufficiently adversely affected by the administration of a noxious stimulus. Electrophysiological recordings from trigeminal nerves identified polymodal nociceptors on the head of the trout with physiological properties similar to those described in higher vertebrates. These receptors responded to mechanical pressure, temperatures in the noxious range (more than 40 °C) and 1% acetic acid, a noxious substance. In higher vertebrates nociceptive nerves are either A-delta or C fibres with C fibres being the predominating fibre type. However, in the rainbow trout A-delta fibres were most common, and this offers insights into the evolution of nociceptive systems. Administration of noxious substances to the lips of the trout affected both the physiology and the behaviour of the animal and resulted in a significant increase in opercular beat rate and the time taken to resume feeding, as well as anomalous behaviours. This study provides significant evidence of nociception in teleost fishes and furthermore demonstrates that behaviour and physiology are affected over a prolonged period of time, suggesting discomfort.

Keywords: nociception; pain; rainbow trout; trigeminal

1. INTRODUCTION

Nociception, the detection of tissue-damaging stimuli, is evident in a number of different phyla including birds and mammals (Walters 1996), but studies on lower vertebrates have suggested a lack of nociceptors and pain perception (e.g. Atlantic stingray (*Dasyatis sabina*); Coggeshall *et al.* 1978; Leonard 1985; or long-tailed stingray (*Himantura fai*); Snow *et al.* 1993). From the perspective of the evolution of sensory function in vertebrates, the study of sensory systems in lower vertebrates is of great interest. Olfactory, gustatory and chemosensory systems have been well described in fishes (Belousova *et al.* 1983; Kotrschal 2000), but relatively little attention has been paid to nociception. The trigeminal nerve, the fifth cranial nerve, innervates the majority of sensory information from the head of vertebrates and as such conveys somatosensory information from potentially damaging stimuli to the brain. A study on the most primitive living vertebrate, the lamprey (*Petromyzon marinus*), suggested that there were trigeminal receptors that responded to burning of the skin (Matthews & Wickelgren 1978). The physiological responses of these receptors, however, were not well characterized and the responses recorded may have been a result of damage to the receptor field rather than the preferential sensitivity to a noxious temperature *per se*. Furthermore, the lamprey lacks myelination, and its closest evolutionary relatives, the elasmobranchs, are deficient in unmyelinated fibres and no nociceptors have been identified (Leonard 1985; Snow *et al.* 1993). A recent study on the rainbow trout (*Oncorhynchus mykiss*) demonstrated that, although most primary afferent somatosensory fibres were A-delta fibres, unmyelinated C fibres were present in the trigeminal nerve (Sneddon 2002). Free nerve endings of A-delta and C fibres act as nociceptors in higher vertebrates and have been well characterized (Lynn 1994) and thus there is the potential for these neurons to act as nociceptors in the rainbow trout.

A number of different classes of nociceptors have been described in mammals but they are commonly slowly adapting mechanoreceptors that preferentially respond to noxious heat (greater than 40 °C) and are termed mechanothermal nociceptors (Lynn 1994). If these nociceptors also respond to noxious chemicals such as bee venom, acid, bradykinins and acetyl choline, then they are classified as polymodal nociceptors (Lynn 1994). Using electrophysiological techniques, nociceptors have been identified in amphibia (Spray 1976), birds (Gentle 1992, 1997; Gentle & Tilston 2000) and mammals (Yeomans & Proudfit 1996) including primates (Kenshalo *et al.* 1989) and humans (Torebjörk & Hallin 1974; Hallin *et al.* 1981). Therefore, if we can demonstrate that the rainbow trout possesses the neural apparatus to detect noxious stimuli, then this will confirm that the trout is capable of nociception, the simple detection of and reflex response to a noxious stimulus (Kavaliers 1988; Bateson 1991). To suggest pain perception, it must be shown that any behavioural or physiological responses are not merely reflexive.

Pain in humans has been defined as an 'unpleasant sensory and emotional experience associated with actual or potential tissue damage' (IASP 1979, p. 249). It is impossible to truly know whether an animal has an emotion because we cannot measure emotion directly. Therefore, emotion does not feature in the definition of pain in

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animals (Zimmerman 1986; Bateson 1991). What an animal 'feels' is possibly nothing like the experience of humans with a more complex brain structure; however, the animal's experience may be unpleasant or cause suffering and their discomfort is no less important in terms of biology or ethics. To examine possible pain perception in an animal, indirect measurements of behavioural and physiological responses to a potentially painful event are made and then we assess the evidence collected from the data, as is routinely done in welfare studies (Bateson 1991; Broom 1991; Gentle 1992; Gonyou 1994; Bradshaw & Bateson 2000; Mason *et al.* 2001; Roughan & Flecknell 2001; Molony *et al.* 2002). If a noxious event has sufficiently adverse effects on behaviour and physiology in an animal and this experience is painful in humans, then it is likely to be painful in the animal.

To demonstrate that an animal is capable of pain perception, it must be shown that, first, the animal can perceive the adverse sensory stimulus and, second, that it reacts both physiologically (e.g. inflammation, cardiovascular changes) and behaviourally (move away from the stimulus). However, to show that this is not simply a nociceptive reflex, it is necessary to show that the animal learns that the stimulus is associated with an unpleasant experience and avoids it. Certainly it has been demonstrated that fishes can learn to avoid an adverse stimulus such as an electric shock (Ehrensing *et al.* 1982) and hooking during angling (Beukema 1970*a*,*b*). Additionally, suffering or discomfort is implicated if the animal's behaviour is adversely affected (Zimmerman 1986). These criteria have been demonstrated to be met in mammals (Roughan & Flecknell 2001), birds (Gentle 1992) and amphibians (Stevens 1992) but not in teleost fishes.

The purpose of the present study was to determine whether nociceptors are present in the trigeminal system on the head of the trout and to determine the physiological and behavioural consequences of prolonged noxious stimulation. Recordings were made from the trigeminal nerve to identify whether nociceptors were present on the face and head of the trout. Behavioural and physiological responses of the fish to administration of acutely algogenic substances to the lips were assessed to examine whether there was the potential for pain perception in this species. The criteria that must be met for animal pain are, first, the demonstration of the sensory capability of detecting potentially painful stimuli, and, second, the performance of adverse behavioural responses to a potentially painful event that are not simple reflexes.

2. METHODS

(a) *Electrophysiological recordings from the trigeminal ganglion*

Rainbow trout (weighing 750 ± 100 g, $n = 10$) were supplied by a commercial fish supplier. The fishes were maintained as described in a previous study (Sneddon 2002). Trout were caught individually by netting and were initially anaesthetized by immersion in MS 222 (50 mg l^{-1}) to facilitate weighing and intraperitoneal injection of Saffan (0.3 ml 100 g^{-1}; Schering-Plough Animal Health, Welwyn Garden City, UK). Once deep anaesthesia was achieved, the fish was placed into a stainless-steel cradle cushioned with wet paper towels and held in position with Velcro straps. The fishes had reached surgical deep-plane anaesthesia, were not conscious and had to be ventilated by flushing fresh water over the gills by means of a tube held in place by a specially constructed mouthpiece. Skin and bone were removed above the brain and then the olfactory and optic lobes and cerebellum were removed via a suction tube connected to a vacuum pump. This procedure is known as decerebration and renders the animal insentient because it is left with only a brainstem. To prevent muscular twitching, Pavulon, a neuromuscular blocker (pancuronium bromide 2 mg ml^{-1}), was injected intramuscularly (0.08 ml 100 g^{-1} fish weight). Bone was removed to expose the trigeminal ganglion and the ganglion was desheathed and covered in paraffin to prevent moisture loss. Glass-insulated tungsten microelectrodes (tip diameter of 10 μm) were used to record from afferent cell bodies. The extracellular action potentials were amplified using a NL100 head stage connected to a NL104 preamplifier (Neurolog System, Digitimer Ltd, UK). The signal was displayed on a storage oscilloscope (5113, Tektronix INC) and stored on a PC using a Micro 1401 interface and Spike2 software (CED, UK).

Neural activity was recorded from single cells in the trigeminal ganglion following the application of stimuli to the head of the fish. A glass mechanical probe (0.1 mm in diameter) was lightly applied to the facial skin in order to locate a receptor field. Once located, the mechanical threshold of the receptor was determined by applying von Frey hairs (0.1–15.0 g at 0.1 g intervals) to the receptor field. The diameter of the receptive field was measured to the nearest 0.1 mm using Vernier calipers. The receptor was then tested for thermal and chemical sensitivity. A thermal stimulator was placed 1 mm above the area of the receptor field so that it did not burn the skin and the stimulator raised the temperature to 58 °C. Thermal sensitivity was determined by heating the skin at a rate of 1 °C s^{-1} up to 58 °C using a prefocused quartz glass light bulb with built-in reflector (A1231, 12 V, 100 W Wotan) orientated vertical to the skin. If the receptor responded to the increase in temperature, the threshold was determined and the response had to be repeatable. Temperature was measured using a type K thermocouple placed in the centre of the bulb focus and was controlled by a feedback circuit. The skin temperature was held at 58 °C for 10 s, after which it rapidly returned to normal. The temperature increase of 1 °C s^{-1} allowed the threshold to be determined. To ascertain chemosensitivity, a drop of 1% acetic acid was placed onto the receptor field. The first 5 ms after the addition of the drop were disregarded as possibly being a response to the touch of the drop; a response to this noxious chemical stimulation was confirmed if the action potentials measured from mechanical and/or thermal stimulation of the receptor fired after this period. Again this response was repeatable. A drop of water was also placed onto the receptive field to act as a control stimulus. None of the receptors responded to this. Conduction velocities were obtained by placing silver wire stimulation electrodes onto the receptor field, and stimulating the receptor directly by an electrical pulse. This stimulated the fibre to produce an action potential and the conduction velocity was determined using the time that the action potential was recorded after the stimulus and the estimated distance travelled from the receptive field to the recording electrode in the trigeminal ganglion.

(b) *Behavioural responses to administration of algogenic substances*

Twenty rainbow trout (weighing 30–100 g) were obtained from a commercial fish supplier and individually housed in rectangular tanks (45 cm × 25 cm × 35 cm) with a constant flow of

water at 11 ± 1 °C and a feeding ring (10 cm in diameter) secured on the water surface at the same location in each tank. One half of the tank was covered by an opaque lid (22.5 cm × 25 cm) to provide an area of shelter, whereas the other half had a transparent lid and this was where the feeding ring was located. Each tank had a gravel substrate and was continuously aerated via an airstone and tubing connected to an air pump. Each fish was trained twice daily, morning and afternoon, to come to the ring to receive food pellets (Trouw Aquaculture, UK) in response to a light cue above the tank (one test equals one trial; mean number of trials to learn ± s.e. was 10 ± 4). Once the fishes had learned to feed at the ring by successfully performing six consecutive trials they received two weeks' further training to ensure that they were truly conditioned to the light stimulus (i.e. responded to light only before food presentation, and they had to perform another 14 trials successfully to be included in the experiment). Fishes were then assigned to one of four treatment groups: (i) saline—0.1 ml sterile saline injected (25 gauge needle and 1 ml syringe) into frontal lips; (ii) venom—0.1 ml bee venom (1 mg ml^{-1} sterile saline) injected into frontal lips; (iii) acid—0.1 ml acetic acid (0.1% in sterile saline) injected into frontal lips; and (iv) control—fish handled but received no injection.

Acetic acid and bee venom were chosen because the protons of the acid stimulate nociceptive nerves in mammals (Martinez *et al.* 1999) and frogs (Hamamoto *et al.* 2000), the venom has an inflammatory effect in mammals (Lariviere & Melzack 1996) and both are known to be painful in humans. Before treatment the behaviour and opercular (gill) beat rate were measured continuously for 15 min. Behaviours recorded were position in the tank (under covered or exposed area) and swimming activity (direct movement of fishes for more than one body length). Fishes were then individually anaesthetized using benzocaine (1.5 ml (50 mg l^{-1} ethanol) l^{-1}) and were carefully injected with the appropriate substance into the upper and lower frontal lips or were handled but not injected. The fishes were in medium- to deep-plane anaesthesia during this procedure and had lost all reflex activity and muscular control. Trout were placed back into their original tanks and allowed 30 min to recover from the anaesthesia. Behaviour and opercular beat rate were recorded for 15 min and then the light was switched on and food subsequently introduced to the tank. If the fish did not respond by swimming to the feeding ring to feed, it was left for a further 30 min, then a further 15 min of observations were recorded and the light cue and food given. This regime continued until the fish resumed feeding. All fishes ingested food within *ca.* 4 h. The times taken to perform the feeding-ring task and resume feeding in each of the four groups were compared using one-way ANOVA. The percentage of time spent in the covered area for each fish in all four groups was determined before and after the treatment and these values were compared using Mann–Whitney *U*-tests. Frequency of swimming activity was calculated for each fish in the experimental groups and before and after the treatment, and these values were also compared using Mann–Whitney *U*-tests.

In a second experiment, six rainbow trout were trained as described above; however, half of these were fed live red mosquito larvae instead of pellets to provide a softer food. All fishes were injected with bee venom and assessed for behaviour and opercular beat rate as already described. The times taken to resume feeding on the two different diets were compared using a Kruskal–Wallis test owing to the low sample size, which was chosen for ethical reasons.

Figure 1. Position of polymodal mechanoreceptors or nociceptors, mechanothermal receptors and mechanochemical receptors on the head and face of the rainbow trout, *Oncorhynchus mykiss* (triangles, polymodal nociceptor; diamonds, mechanothermal nociceptor; hexagons, mechanochemical receptor).

All the fishes used in both experiments were held for a further 3 days and trained in the conditioning task twice a day. All fishes continued to perform the task successfully and to ingest food; therefore, there appeared to be no chronic effects on associative learning and appetite. At the end of the 3 days, the trout were individually killed by an overdose of anaesthetic.

3. RESULTS

(a) *Characterization of nociceptors*

We located 58 receptors on the face and head of the rainbow trout. Twenty-two of these receptors could be classified as nociceptors (figure 1) as they responded to mechanical pressure by a slowly adapting firing pattern and were also stimulated by noxious heat (more than 40 °C), and out of these, 18 also responded to algogenic chemical stimulation (1% acetic acid; figure 2*a–c*). The response of the receptors to mechanical, noxious thermal and chemical stimulation clearly characterizes them as polymodal nociceptors (table 1). There were four receptors that did not respond to chemical stimulation and are classified as mechanothermal nociceptors. A third group of receptors ($n = 6$) responded to only mechanical and chemical stimulation, but without a detailed investigation of their physiological characteristics they cannot be classified as nociceptors at present and are referred to as mechanochemical receptors. A further 16 receptors gave a slowly adapting response to mechanical stimulation and another 14 receptors gave a rapidly adapting response, but none of these responded to thermal or chemical stimulation and they are possibly pressure and touch receptors, respectively (Sneddon 2003). The characteristics of the polymodal and mechanothermal nociceptors and the mechanochemical receptors are shown in table 1. Mechanical thresholds of the three types ranged between 0.1 and 7.1 g and conduction velocities were recorded between 0.97 and 8.5 m s^{-1}. Out of all the polymodal nociceptors that were recorded from, only one was an unmyelinated C fibre and the rest were A-delta fibres.

Table 1. Characteristics of the three types of receptor found on the head of the rainbow trout. Values shown are means ± s.e.

	polymodal nociceptors ($n = 18$)	mechanothermal nociceptors ($n = 4$)	mechanochemical receptors ($n = 6$)
diameter of receptor (mm)	3.20 ± 0.4	2.83 ± 1.0	2.52 ± 0.4
mechanical threshold (g)	0.83 ± 0.4	0.1 ± 0.0	0.78 ± 0.53
thermal threshold (°C)	49.3 ± 1.4	46.2 ± 2.4	none
acid response	yes	none	yes
conduction velocity ($m\ s^{-1}$)	3.96 ± 0.4	3.71 ± 0.5	4.28 ± 0.1

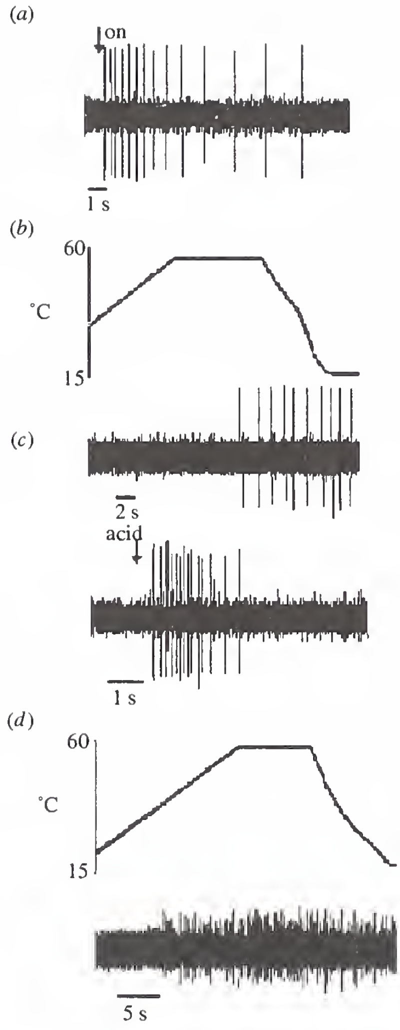

Figure 2. A polymodal nociceptor responding to (*a*) mechanical, (*b*) thermal and (*c*) chemical stimulation (1% acetic acid). The receptor (*a*) adapts slowly to mechanical stimulation ('on' indicates application of the stimulus), (*b*) has a thermal threshold of 58 °C and (*c*) responds to application of a drop of acetic acid onto the receptive field. (*d*) A polymodal nociceptor with a thermal threshold of 42.3 °C.

Thermal responses were seen only above 40 °C and thresholds ranged from 40 °C to 58 °C (figure 2*b*,*d*). The diameter of the receptor field ranged from 1.6 mm to 9 mm × 1 mm. Interestingly, we found no thermal receptors that responded to temperature in the range 20 °C to 40 °C.

(b) *Behavioural and physiological responses to acute noxious stimulation*

Significant increases in opercular beat rate were found in all four groups after the treatment (control and saline: *ca.* 52 beats min^{-1} before to 70 beats min^{-1} after treatment) although the venom and acid groups had the most significantly elevated rates (*ca.* 52 beats min^{-1} before to 93 beats min^{-1} after treatment; figure 3*a*; $F_{3,16} = 27.52$, $p < 0.001$). This physiological effect was coupled with profound effects on the fishes' behaviour. It took control and saline fishes *ca.* 80 min to begin ingesting food again whereas venom and acid fishes took *ca.* 170 min (figure 3*b*; $F_{3,16} = 7.29$, $p = 0.003$). In addition to this, we performed the second experiment, which tested whether the fishes would resume feeding more quickly if fed on a softer foodstuff, but there was no significant difference in the time taken to resume feeding ($H = 0.05$, $p = 0.827$, d.f. = 1).

Activity levels were not affected by the treatment whether it was potentially painful ($W = 130.5$, $p = 0.057$) or not ($W = 107.0$, $p = 0.908$; median frequency before $= 0.356\ min^{-1}$; after $= 0.326\ min^{-1}$) although there was a trend for the venom- and acid-injected fishes to reduce the amount of swimming activity (median frequency before $= 0.935\ min^{-1}$; median frequency after $= 0.265\ min^{-1}$). Position in tank or use of the sheltered area was also not affected by the noxious injections ($W = 103$, $p = 0.910$; median percentage time spent under cover before = 53.3%; after = 55.8%) or the control treatments ($W = 106$, $p = 0.970$; before = 53.9%; after = 63.0%). Observations following acid and venom injection showed that the fishes performed anomalous behaviours after the treatment that were not seen in the control or saline groups: acid and venom fishes performed 'rocking' where the fishes moved from side to side balancing on either pectoral fin while resting on the gravel (mean frequency $0.37\ min^{-1}$ for the venom group and $0.45\ min^{-1}$ for the acid group). The acid group was also observed to rub their lips into the gravel and against the tank walls but the venom group did not perform this behaviour.

4. DISCUSSION

The polymodal nociceptors found here in the trout have similar properties to those found in amphibians (Stevens 1992), birds (Gentle 1992, 1997) and mammals (Handwerker *et al.* 1987) including humans (Lynn 1994). Nociceptors, by definition, preferentially respond to noxious injurious stimuli and this study demonstrates that the

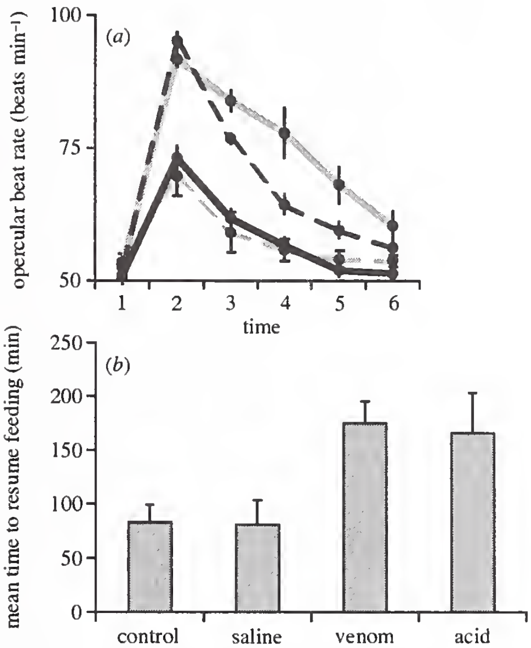

Figure 3. (*a*) Mean (± s.e.m.) opercular beat rate of each treatment group 20 min before treatment and at each observation afterwards (time 1 is 20 min before treatment, time 2 is 30 min after treatment and each time point after this is *ca.* 30 min after the previous point). (Grey dashed line, control; black solid line, saline; grey solid line, venom; black dashed line, acid.) (*b*) The mean (± s.e.m.) time taken for fishes in each treatment group to resume ingesting food after the treatment.

rainbow trout is capable of nociception (Kavaliers 1988; Bateson 1991). Receptor diameter, thermal thresholds and mechanical responses are similar to those measured in higher vertebrate groups (Torebjörk & Hallin 1974; Spray 1976; Hallin *et al.* 1981; Kenshalo *et al.* 1989; Yeomans & Proudfit 1996; Gentle & Tilston 2000). Mechanical thresholds were lower than those found in humans: at least 0.6 g is required for noxious stimulation in human skin (Lynn 1994) but many of the nociceptors in the fish skin were stimulated by 0.1 g. This may be a consequence of the more easily damaged nature of the fish skin requiring the nociceptors to have lower thresholds. Similar thresholds were found in mammalian eye nociceptors (Belmonte & Gallar 1996). However, fish nociceptors have comparable mechanical thresholds to those found in the mammalian eye.

None of the trigeminal receptors in this study was stimulated by temperatures in the range 20 °C to 40 °C. A number of studies have demonstrated a lack of thermal receptors in invertebrates and other lower vertebrates (Matthews & Wickelgren 1978; Leonard 1985; Walters 1996). This suggests that thermal receptors in the non-noxious range potentially evolved in vertebrate groups that lead a more terrestrial existence. These thermal receptors may have evolved in response to temperature fluctuations in the terrestrial environment. It is unlikely that the rainbow trout would come into contact with such high noxious heat as used in this study as this species inhabits waters below 25 °C. The nociceptors of this fish respond only above 40 °C and this is typical of nociceptors in higher vertebrates. This would suggest that either in the distant evolutionary past the animals encountered temperatures above 40 °C, or the response to such high temperatures is a fundamental physiological mechanism or property of nociceptive nerve endings, as has been demonstrated in rat cultured dorsal root ganglion neurons (Lyfenko *et al.* 2002). These dorsal root neurons would also not come into direct contact with noxious temperatures, but they are responsive only to temperatures in the noxious range. It would be interesting from a comparative point of view to assess nociceptive responses in a tropical fish species because they would encounter higher temperatures. The mechanochemical receptors did not respond to thermal stimulation and cannot be classified as nociceptors. Further work is required to test these receptors with a variety of chemicals to ascertain whether they are simply chemoreceptors, or, if they are nociceptive, they respond only to noxious chemicals.

Assessing the subjective experiences of animals plays an increasingly large role in animal welfare (Broom 1991; Gentle 1992; Dawkins 1998; Bradshaw & Bateson 2000; Mason *et al.* 2001). To date, little attention has been paid to potential pain perception in fishes. In our behavioural experiments, we trained fishes to come to a feeding ring in response to a light cue and then assigned them to one of four treatment groups; three of these groups had bee venom, acetic acid or saline injected into the lips and the fourth group was simply a handled control. After injection of algogenic substances, the resulting increase in opercular rate is similar to that recorded when trout are swimming at maximum speed (Altimiras & Larsen 2000) and the rate is much greater than the rate recorded after handling stress (increase to a maximum of 69 beats min^{-1}; Laitinen & Valtonen 1994). The control and saline groups showed similar increases in opercular beat rate to stressed fishes (Laitinen & Valtonen 1994) and this is probably the result of the handling and anaesthetic procedure. Respiratory changes have been demonstrated in mammals and humans enduring a nociceptive event (Kato *et al.* 2001) and so this dramatic rise in ventilation rate may be a physiological response to noxious stimulation in the rainbow trout.

The rainbow trout injected with acetic acid or bee venom performed anomalous behaviours that were not performed by the saline or control groups. Rocking behaviour was seen in both venom and acid treatment groups and this behaviour was performed only in the 1.5 h after injection. This is reminiscent of the stereotypical rocking behaviour of primates that is believed to be an indicator of poor welfare and thought to be performed as a comfort behaviour (Gonyou 1994). The performance of anomalous behaviours usually occurs within a short time period after the occurrence of a painful event when the pain is most intense (Molony *et al.* 2002). Only the acid group performed rubbing of the lips against the gravel and the sides of the tank. The act of rubbing an injured area to ameliorate the intensity of pain has been demonstrated in humans and in mammals (Roveroni *et al.* 2001). Overall, the administration of noxious substances had a negative effect on the fishes' behaviour. To our knowledge, the performance of these behaviours has not been observed in fishes before. These behaviours may be indicative of dis-

comfort and may have a potential use as indicators of pain or the occurrence of a noxious event in fishes. However, in humans and other animals pain is a specific experience and each different type of pain may have different behavioural responses and may also be species specific (Kavaliers 1988). Therefore, further studies should target noxious stimulation of other areas of the fish body to assess whether the behaviours seen in this study are universal.

The venom and acid injected fishes took *ca.* 3 h to resume ingesting food, whereas the saline and control groups took *ca.* 1 h. The venom and acid groups may be experiencing discomfort and so take longer to perform the task and resume feeding. This may be similar to guarding behaviour, where an animal avoids using a painful limb to prevent more pain and damage being caused to the affected area (Gentle 1992). Handling and anaesthesia are known to be stressful, causing an elevation in respiration rate (Laitinen & Valtonen 1994), and would account for the delay in the saline and control groups performing the conditioning task. Giving the noxiously stimulated trout softer food did not affect the time taken to begin feeding again. Therefore, it appears that the rainbow trout does not feed when affected by the administration of a noxious agent to the lips and resumes feeding only when the behavioural and physiological effects subside.

Our results demonstrate that the rainbow trout possesses nociceptors that detect noxious stimuli and that both the behaviour and the physiology of the rainbow trout are adversely affected by stimuli known to be painful to humans. The behaviours shown by the trout after injection of a noxious stimulus are complex in nature and as such may not be simple reflexes. The performance of rocking behaviour and rubbing of the affected area, possible indicators of discomfort, suggest that higher processing is involved in the behavioural output and this is similar to some of the responses of higher vertebrates (Gonyou 1994; Roughan & Flecknell 2001) and man (Kato *et al.* 2001) to noxious stimuli. Other behavioural studies have shown that fishes learn to avoid aversive noxious events such as an electric shock but fishes that had morphine, an analgesic, administered failed to learn to avoid the electric shock (Ehrensing *et al.* 1982). Together, these electrophysiological and behavioural results show that the rainbow trout has a well-developed nociceptive system. Previous anatomical studies have suggested that marine elasmobranchs do not have nociceptors (Leonard 1985; Snow *et al.* 1993). This may represent an evolutionary divergence between the teleost and elasmobranch lineages.

Interestingly, there is a higher percentage of A-delta fibres (25%) in the trigeminal nerve than C fibres (4%; Sneddon 2002) and the majority of nociceptors were recorded from A-delta fibres. Only one out of the 18 nociceptors we recorded from had a conduction velocity in the range of C fibre velocity ($0.97\ \mathrm{m\ s^{-1}}$) and the rest were A-delta fibres. Studies in mammals have stressed the importance of C fibres in prolonged nociceptive stimulation because they act as polymodal nociceptors; A-delta fibres, being mechanothermal nociceptors, participate only in acute short-term responses usually to alert the nervous system to immediate injury (Matzner & Devor 1987; Lynn 1994; Gentle 1997). However, A-delta fibres predominate in the rainbow trout and the behavioural effects of a noxious stimulus, such as bee venom, were prolonged over *ca.* 3 h. Therefore, in teleosts, A-delta fibres potentially have a dual role in mediating reflex escape behaviour and prolonged noxious stimulation, whereas in higher vertebrates, C fibres may have evolved to become more numerous and have a more prominent function in prolonged noxious stimulation and inflammatory pain. More detailed electrophysiological recordings on A-delta fibres in the trout are necessary to confirm this hypothesis. Sneddon (2002) suggested that the higher proportion of C fibres in the higher vertebrates compared with the teleost was due to the advance onto land during evolution and the increased chance of injury resulting from gravity, extremes of temperature and noxious gases. The aquatic environment provides buoyancy, dilution of chemicals and a relatively stable thermal environment and so perhaps teleosts have not dedicated such a great amount of neural wiring to nociception as have terrestrial vertebrates.

The results of the present study demonstrate nociception and suggest that noxious stimulation in the rainbow trout has adverse behavioural and physiological effects. This fulfils the criteria for animal pain as stated in § 1. Future work should examine the cognitive aspects of noxious stimulation to assess how important enduring a noxious potentially painful event is to the mental well-being of this species.

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2.1.

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The Neurobehavioral Nature of Fishes and the Question of Awareness and Pain

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ABSTRACT: This review examines the neurobehavioral nature of fishes and addresses the question of whether fishes are capable of experiencing pain and suffering. The detrimental effects of anthropomorphic thinking and the importance of an evolutionary perspective for understanding the neurobehavioral differences between fishes and humans are discussed. The differences in central nervous system structure that underlie basic neurobehavioral differences between fishes and humans are described. The literature on the neural basis of consciousness and of pain is reviewed, showing that: (1) behavioral responses to noxious stimuli are separate from the psychological experience of pain, (2) awareness of pain in humans depends on functions of specific regions of cerebral cortex, and (3) fishes lack these essential brain regions or any functional equivalent, making it untenable that they can experience pain. Because the experience of fear, similar to pain, depends on cerebral cortical structures that are absent from fish brains, it is concluded that awareness of fear is impossible for fishes. Although it is implausible that fishes can experience pain or emotions, they display robust, nonconscious, neuroendocrine, and physiological stress responses to noxious stimuli. Thus, avoidance of potentially injurious stress responses is an important issue in considerations about the welfare of fishes.

KEY WORDS: pain, nociception, stress, awareness, anthropomorphism.

I. INTRODUCTION

In recent years, commercial and sport fishing have been challenged increasingly on grounds of humaneness. These challenges have appeared in the research literature (Verheijen and Flight, 1997) and in the arena of public opinion (American Fisheries Society Position Statement, 1999). A principal underlying assumption of the scientific and nonscientific challenges is that fishes are capable of suffering from pain in a manner similar to that experienced by humans (Bateson, 1992; Gregory, 1999; Verheijen and Flight, 1997). A similar assumption is evident in federal regulations pertaining to the use of fishes in research, in that these regulations apply to all live vertebrate animals (National Institutes of Health, 1985; National Research Council, 1996). An examination of the validity of this assumption from a scientific perspective is clearly needed.

Advances in neuroscience research during recent decades have greatly improved our understanding of the neurological basis of pain as well as the neurobiological nature of fishes. The objective of this article is to utilize this knowledge to examine the neurobehavioral nature of fishes and to specifically address the question of whether fishes are capable of experiencing pain and suffering. In order to achieve this objective, the article addresses the following issues and concepts:

1. Anthropomorphic thinking undermines our understanding of other species.

2. An evolutionary perspective is essential to understanding the neurobehavioral differences between fishes and humans.

3. Neurobehavioral differences between fishes and humans result from known differences in central nervous system structure.

4. Behavioral responses to noxious stimuli are separate from the psychological experience of pain, such that behavioral responses to these stimuli can occur in the absence of pain experience.

5. Awareness of pain in humans depends on specific regions of the cerebral cortex. Fishes lack these brain regions and thus lack the neural requirements necessary for pain experience.

6. Previous assertions that fishes can experience pain will be critiqued.

7. Conscious experience of fear, similar to pain, is a neurological impossibility for fishes.

8. Fishes display robust but nonconscious, neuroendocrine, and physiological stress responses to noxious stimuli. Potentially injurious stress responses, as opposed to pain or emotional distress, are the proper matter of concern in considerations about the welfare of fishes.

II. ANTHROPOCENTRISM HINDERS UNDERSTANDING OF OTHER SPECIES

A critical guiding principle for understanding the nature of vertebrates with differing evolutionary histories such as fishes and mammals are to view them from the perspective of their respective evolutionary paths. The evolutionary radiation of modern fishes unfolded, to a great extent, separately from that of mammals (Long, 1995). Consequently, comparative neuroanatomical studies have revealed that the mammalian brain has many embellishments and expansions that are not present or present only in more elemental form in fishes (Butler and Hoods, 1996; Nieuwenhuys *et al.*, 1998a). These developments of the mammalian, especially the human brain, have engendered neurobehavioral and psychological capacities that are not present in fishes. Thus, human capacities such as language and consciousness with self-awareness have resulted from later, very separate evolutionary development of this uniquely complex and enlarged brain (Deacon, 1992a; 1992bc; Donald, 1991; Lieberman, 1992; Macphail, 1998; Preuss, 2000; Raichle, 2000). The existence of these complex capacities in humans is not a justification for presuming the existence of similar capacities in species that had very different evolutionary histories leading to very different neurobehavioral adaptations. Recognition of this basic difference in evolutionary histories is essential to a valid understanding of the neurobehavioral differences between ourselves and other vertebrates. This evolutionary perspective is critical because of the widespread anthropomorphic tendency of humans to view other vertebrates as having mental states similar to our own. So, in

observing the actions of other organisms where the actions appear to resemble our own, often it is assumed that these non-human organisms have intentions and experiences similar to ours. This human tendency to attribute mental states to others is called "theory of mind" and is probably the basis for our tendency to feel empathy toward other people. Theory of mind is thought to have evolved as a device for increasing our accuracy in predicting the behavior of other humans (Bogdan, 2000; Macphail, 1998). If we anthropomorphically apply the human theory of mind to other organisms, however, we are increasingly likely to be mistaken as the neuropsychological differences between ourselves and another organisms increases. Most scientists familiar with higher nervous system functions would not attribute human mental states and experiences to an earthworm or an ameba. However, the matter is more troublesome when we are observing the behavior of other vertebrates, especially mammals, where we are often inclined to interpret their behavior to represent the occurrence of human-like mental states (Kennedy, 1992). This problem seems especially great in cases where pain-like behavior is involved.

An additional basic problem with anthropomorphism is that the behavior and underlying brain systems of vertebrates are highly diverse and for many species highly distinctive. Consequently, there are many vertebrate neurobehavioral systems and refinements that have no counterpart in humans. Examples would be electroreception and signaling by electric fish or echolocation in bats, dolphins, and some birds. Likewise, many human behaviors and their underlying brain systems are profoundly unique. Human uniqueness is evident in our capacity to learn and create languages spontaneously, our great behavioral diversity, our capability for long-term planning, our creativity, including art, science, and the existence of religious beliefs. The massive expansion of the human cerebral cortex appears to be at the heart of this uniqueness. Given the extreme distinctiveness of humans in so many respects, it is highly inappropriate and misleading to use human nature as the basis for generalizing to other, decidedly different, species. This matter is no different from using the physiological mechanisms underlying respiratory or water/ion-balance functions of fishes as an unqualified basis for inferring the mechanisms for breathing or water/ion balance in mammals.

The quandary of the private nature of personal experience has a practical, empirical solution. This solution lies in one of the most well-established principles of neuroscience: that neurobehavioral function, including sensory perception and psychological experience, are based on specific, identifiable properties of nervous system structure. This principle is most obvious for sensory and motor function. Sensory regions of the cerebral cortex show pronounced species differences in macroscopic and microscopic structure (and associated physiological properties) that are responsible for species differences in sensory function. For example, echolocating bats have highly specialized regions of auditory cortex for processing echoes received from self-generated vocalizations. Such cortical regions are absent in the brains of mammals without echolocation capability (Suga and Kanwal, 1995). In a similar vein, the motor cortex of humans has an enlarged region devoted to the control of the fingers and thumb. This degree of functional expansion is not present in species having forepaws rather than hands, opposable thumbs, and the associated manual dexterity of humans (Allman, 1999; Kandel *et al.*, 2000). Even though the neurostructural basis for some of the novel human dimensions of nervous system function, such as language or long-term planning, have not been delineated to the same degree as many aspects of sensory and motor function, the basic structural substrates of these capabilities are well known (Kolb and Whishaw, 1995). The important point for the purpose this discussion is that an examination of the nervous system, especially brain structural organization, provides powerful insights into the nature of an organism, including its capabilities and limits.

III. THE FUNCTIONAL PLANS AND CAPABILITIES OF NERVOUS SYSTEMS ARE BEST UNDERSTOOD FROM THE PERSPECTIVE OF THEIR EVOLUTIONARY DEVELOPMENT

The vertebrate nervous system evolved interdependently with the vertebrate body plan. Throughout this process, certain features of the vertebrate nervous system have been perpetuated, although

the evolutionary process has mainly resulted in progressively more complex brains. Some of the most profound evolutionary changes in early vertebrates, such as the acquisition of jaws, appear rather simple on the surface. However, such evolutionary changes necessitated corresponding neural adaptations that led to far-ranging neurobehavioral diversification in foods and feeding styles that allowed exploitation of more diverse physical environments (Allman, 1999; Long, 1995; Radinsky, 1987). As organisms made the transition from a purely aquatic existence to a terrestrial one, new neurological and behavioral adaptations allowed exploitation of different modes of life. There have been many major additions and refinements, especially in the mammalian central nervous system, in response to natural selection for niches very different from those occupied by fishes. Examples are special adaptations for controlling the limbs for terrestrial locomotion and grasping, the function of body hair as a tactile sensory system, neuroendocrine adaptations for lactation and autonomic nervous system control of sweat glands for thermoregulation. The great demands of mammalian endothermy were supported by the evolution of the energy-conserving, forebrain-controlled, quiet sleep state, which occurs in its most well-developed and highly differentiated form in mammals (Karmanova, 1982; Nicolau *et al.*, 2000). Thus, there have been changes in neural and behavioral complexity during vertebrate evolution in which the nervous system, with its stable genetic foundation has undergone great modification, elaboration, and diversification.

The presence of an obvious neural structure-function association between differing modes of vertebrate existence has a corollary principle: common functional problems tend to have common solutions, whereas special functional problems tend to have more specialized, neurostructurally conspicuous solutions. For example, the neural control of breathing is mediated by evolutionarily conserved neural systems in the lower brainstem of all vertebrates, in spite of the differences in the of manner of breathing that exists between fishes, mammals, and other vertebrates (Wullimann, 1998; Kandel *et al.*, 2000). Similarly, the neural systems controlling locomotor movements are present in the spinal cords of fishes and mammals, alike, in spite of the great differences in the specifics and complexity of locomotion in these taxa (Grillner *et al.*, 1998; Kandel *et al.*, 2000; Rose *et al.*, 2000). In the case of brainstem structure, there is a stable genetic foundation, exemplified by hox genes (Allman, 1999; Kandel *et al.*, 2000), that has fostered the evolutionary solution of common problems by progressive modification of conserved, preexisting neural mechanisms. In contrast, a novel functional capability of a species or related group of species typically has a neurostructural solution that is not shared with other species. This is seen in the mammary glands of mammals, where the milk letdown reflex is controlled by a specialized sensory, neuroendocrine, neuromuscular, and glandular system that is not present in non-mammals even though it is derived from characteristics, such as the neurohypophyseal hormone oxytocin, that had a precursor in the brains of ancestral vertebrates (Moore, 1992).

IV. THE CAPACITY FOR CONSCIOUSNESS DEPENDS ON FUNCTIONS OF THE NEOCORTEX, A BRAIN STRUCTURE UNIQUE TO MAMMALS

The question of whether non-human animals, particularly fishes, are consciously aware of experiences is an issue central to this review, for the simple reason that consciousness is a prerequisite to the experience of pain and its distressing emotional aspect. Without consciousness, there is no awareness of pain, which is why anesthetics are used to prevent pain during surgery. It is of interest to note that anesthetic dosages that obtund awareness of pain do not necessarily prevent motor reactions to surgery, a reason that myoneural blocking agents are used as adjuncts to anesthetics (Taylor, 1990). These points, that pain experience depends on conscious awareness and that awareness of pain and bodily responses to injury are separate phenomena, are pursued at greater length subsequently.

An answer to the question of consciousness in non-humans can be found in the differences in brain structure across species. Although vertebrates have a common mode of nervous system

organization, there are great differences across vertebrate taxa in the structure and complexity of the brain (Butler and Hodos, 1996; Nieuwenhuys *et al.*, 1998a). A principal difference between mammalian brains and those of other vertebrates is the expansion and complexity of the mammalian cerebral hemispheres (Nieuwenhuys *et al.*, 1998a; Preuss, 2000; van Dongen, 1998). Cerebral hemisphere size and complexity has reached its greatest extreme in mammals, with the development of neocortex, a six layered type of cortex present only in mammals (Allman, 1999; Preuss, 2000; Voogd *et al.*, 1998). Even among mammals there is a wide range of cerebral hemisphere development, with primates tending to have substantially more neocortex relative to their body size than most other mammals (van Dongen, 1998; Voogd *et al.*, 1998). Humans have the greatest degree of cerebral hemisphere development in two major respects: relatively more total neocortex for our body weight and more differentiation of neocortex structural subtypes (Mountcastle, 1998; Preuss, 2000). The latter fact is especially evident in the large, specialized cortical regions that mediate our distinctive, species-specific capacities for language, long-term planning, and abstract thinking (Donald, 1991; Kolb and Whishaw, 1995; Preuss, 2000).

Extensive evidence demonstrates that our capacity for conscious awareness of our experiences and of our own existence depends on the functions of this expansive, specialized neocortex. This evidence has come from diverse sources such as clinical neuropsychology (Kolb and Whishaw, 1995), neurology (Young *et al.*, 1998; Laureys *et al.*, 1999, 2000a-c), neurosurgery (Kihlstrom *et al.*, 1999), functional brain imaging (Dolan, 2000; Laureys *et al.*, 1999, 2000a-c), electrophysiology (Libet, 1999) and cognitive neuroscience (Güzeldere *et al.*, 2000; Merikle and Daneman, 2000; Preuss, 2000). A strong case has been made that it is mainly those cortical regions that have achieved such massive expansion in humans that are most centrally involved in the production of consciousness (Edelman and Tononi, 2000; Laureys *et al.*, 1999, 2000a-c).

Although consciousness has multiple dimensions and diverse definitions, use of the term here refers to two principal manifestations of consciousness that exist in humans (Damasio, 1999; Edelman and Tononi, 2000; Macphail, 1998): (1) "primary consciousness" (also known as "core consciousness" or "feeling consciousness") and (2) "higher-order consciousness" (also called "extended consciousness" or "self-awareness"). Primary consciousness refers to the moment-to-moment awareness of sensory experiences and some internal states, such as emotions. Higher-order consciousness includes awareness of one's self as an entity that exists separately from other entities; it has an autobiographical dimension, including a memory of past life events; an awareness of facts, such as one's language vocabulary; and a capacity for planning and anticipation of the future. Most discussions about the possible existence of conscious awareness in non-human mammals have been concerned with primary consciousness, although strongly divided opinions and debate exist regarding the presence of self-awareness in great apes (Macphail, 1998). The evidence that the neocortex is critical for conscious awareness applies to both types of consciousness. Evidence showing that neocortex is the foundation for consciousness also has led to an equally important conclusion: that we are unaware of the perpetual neural activity that is confined to subcortical regions of the central nervous system, including cerebral regions beneath the neocortex as well as the brainstem and spinal cord (Dolan, 2000; Güzeldere *et al.*, 2000; Jouvet, 1969; Kihlstrom *et al.*, 1999; Treede *et al.*, 1999).

Although consciousness has been notoriously difficult to define, it is quite possible to identify its presence or absence by objective indicators. This is particularly true for the indicators of consciousness assessed in clinical neurology, a point of special importance because clinical neurology has been a major source of information concerning the neural bases of consciousness. From the clinical perspective, primary consciousness is defined by: (1) sustained awareness of the environment in a way that is appropriate and meaningful, (2) ability to immediately follow commands to perform novel actions, and (3) exhibiting verbal or nonverbal communication indicating awareness of the ongoing interaction (Collins, 1997; Young *et al.*, 1998). Thus, reflexive or other stereotyped responses to sensory stimuli are excluded by this definition. Primary consciousness appears to depend greatly on the functional integrity of several cortical regions of

the cerebral hemispheres especially the "association areas" of the frontal, temporal, and parietal lobes (Laureys *et al*., 1999, 2000a-c). Primary consciousness also requires the operation of subcortical support systems such as the brainstem reticular formation and the thalamus that enable a working condition of the cortex. However, in the absence of cortical operations, activity limited to these subcortical systems cannot generate consciousness (Kandel *et al.,* 2000; Laureys *et al*., 1999, 2000a; Young *et al.,* 1998). Wakefulness is not evidence of consciousness because it can exist in situations where consciousness is absent (Laureys *et al*., 2000a-c). Dysfunction of the more lateral or posterior cortical regions does not eliminate primary consciousness unless this dysfunction is very anatomically extensive (Young *et al.,* 1998).

Higher-order consciousness depends on the concurrent presence of primary consciousness and its cortical substrate, but the additional complexities of this consciousness require functioning of additional cortical regions. For example, long-term, insightful planning of behavior requires broad regions of the "prefrontal" cortex. Likewise, awareness of one's own bodily integrity requires activity of extensive regions of parietal lobe cortex (Kolb and Whishaw, 1995). In general, higher-order consciousness appears to depend on fairly broad integrity of the neocortex. Widespread degenerative changes in neocortex such as those accompanying Alzheimer's disease, or multiple infarcts due to repeated strokes, can cause a loss of higher-order consciousness and result in dementia, while the basic functions of primary consciousness remain (Kandel *et al*., 2000; Kolb and Whishaw, 1995).

The reasons why neocortex is critical for consciousness have not been resolved fully, but the matter is under active investigation. It is becoming clear that the existence of consciousness requires widely distributed brain activity that is simultaneously diverse, temporally coordinated, and of high informational complexity (Edelman and Tononi, 1999; Iacoboni, 2000; Koch and Crick, 1999; 2000; Libet, 1999). Human neocortex satisfies these functional criteria because of its unique structural features: (1) exceptionally high interconnectivity within the neocortex and between the cortex and thalamus and (2) enough mass and local functional diversification to permit regionally specialized, differentiated activity patterns (Edelman and Tononi, 1999). These structural and functional features are not present in subcortical regions of the brain, which is probably the main reason that activity confined to subcortical brain systems can't support consciousness. Diverse, converging lines of evidence have shown that consciousness is a product of an activated state in a broad, distributed expanse of neocortex. Most critical are regions of "association" or homotypical cortex (Laureys *et al*., 1999, 2000a-c; Mountcastle, 1998), which are not specialized for sensory or motor function and which comprise the vast majority of human neocortex. In fact, activity confined to regions of sensory (heterotypical) cortex is inadequate for consciousness (Koch and Crick, 2000; Lamme and Roelfsema, 2000; Laureys *et al*., 2000a,b; Libet, 1997; Rees *et al*., 2000).

V. FISHES HAVE A NEUROBEHAVIORAL NATURE, DIFFERENT IN MANY WAYS FROM MAMMALS, THAT IS CLOSELY TIED TO PHYLOGENETIC DEVELOPMENT OF THEIR CENTRAL NERVOUS SYSTEMS

Fishes are the most ancient type of vertebrate for which fossil evidence is available, dating from the jawless ostracoderms about 500 million years ago (Radinsky, 1987 Long, 1995). Jawed fishes appear in the fossil record more than 400 million years ago. The brain development of the early ostracoderms was extremely simple (Janvier, 1993), resembling that found in modern jawless fishes such as lampreys and hagfish. Nonetheless, the basic pattern of vertebrate brain organization was evident in ostracoderms, which were probably capable of only a very limited behavioral repertoire. Subsequently, fishes have developed into the most diverse vertebrate radiation, comprising more than 25,000 extant species of jawless, cartilaginous, and bony taxa, presenting extreme variety in anatomical, physiological, and behavioral adaptations (Helfman *et al*., 1997). It is critical to recognize that the vertebrate line that gave rise to mammals diverged from the

line that led to extant fishes in the Devonian era, roughly 400 million years ago. The first mammals did not appear until the Triassic, about 200 million years later. Especially important is the fact that teleosts, the most numerous of modern fishes, did not emerge until the Jurassic, long after the advent of mammals. This point further emphasizes the evolutionary separateness of fishes and mammals.

The complexity of fish behavior and associated sensory and motor function varies greatly, with agnathans such as lampreys having more rudimentary or restricted capacities and teleosts being more advanced, with corresponding neural specializations. References pertaining to the following description of behavior can be found in reviews by Davis and Northcutt (1983), Evans (1998), Helfman *et al.* (1997) and Nieuwenhuys *et al.* (1998a).

Sensory capacities of fishes show wide differences in degree of development. Some species, such as salmonids, have color vision and high acuity, whereas benthic species are typically more limited in their response to photic stimuli due to adaptations for life under low light of more limited spectral properties. Chemosensory properties show extreme variations (Sorensen and Caprio, 1998). Some species, such as silurid catfish, have taste receptors across their entire body surface rather than just the oral cavity. Likewise, the structure and function of the olfactory system shows great diversity across species. Feeding behavior is extremely diverse and specialized in the sensory modalities used to locate food and in the oral motor specializations employed in food capture or ingestion. Some of the most distinctive sensorimotor specialists are gymnotid and mormyrid teleosts, electric fish that generate and detect pulsed electrical currents to communicate with their conspecifics and to locate objects in their environment. Reproductive behavior is highly diverse. Most species display little or no parental care, whereas some exhibit active protection of young, as in mouth brooding cichlids or defense of nest sites by male fathead minnows. Social displays in mating can entail elaborate courtship and in some cases, as in sex-changing wrasses, a bisexual behavioral capability.

Fishes are clearly capable of associative learning, that is learning a relationship between sensory stimuli or between a stimulus and a behavioral response. Examples of these types of learning in fish include Pavlovian conditioning of visceral responses such as heart rate and respiration, migration guided by olfactory imprinting, and approach or avoidance conditioning. Although most behavior of fishes is not dependent greatly on learning, it is clear that learning and experience are often necessary for the complete development of species-typical behaviors. A critical point concerning the associative learning shown by fishes is that this form of learning, also known as implicit learning, is a virtually universal capability of vertebrate and invertebrate animals, including those lacking brains (Macphail, 1998). Furthermore implicit learning occurs without conscious awareness, even in humans (Kolb and Whishaw, 1995; Macphail, 1998), so the presence of this type of learning in fishes does not constitute a capacity for awareness.

Unlike the subsequent course of mammalian emergence, the evolution of fishes placed them in a niche where neurobehavioral processes that could be mediated by a brain comprised mainly of brainstem structures and relatively small cerebral hemispheres was adequate for their fitness needs. Fishes, being a highly diverse taxon, have extreme variations in brain structure, especially at the brainstem level (Nieuwenhuys *et al.*, 1998b; Butler and Hodos, 1996). It is important to emphasize that fish brains can have highly complex, unique adaptations due to their diverse evolutionary paths. Consequently, these brains are not merely simpler versions of mammalian (or amphibian or reptilian) brains (Nieuwenhuys *et al.*, 1998b; Butler and Hodos, 1996; Wulliman, 1998). In spite of the diversity and complexity among species, the behaviors of fishes are nonetheless highly stereotyped and invariant for a given species. Such stereotyped species-typical behaviors, however, are not simple or merely reflexive. Species-typical behaviors in vertebrates are known to be controlled by motor patterning mechanisms that are far more complex than reflexes (Ewert, 1987; Fentress, 1987). The basic behaviors involved in reproduction, feeding and drinking, escape or defense, and reactions to noxious stimuli, are controlled by motor patterning mechanisms

that are located mainly in the brainstem and spinal cord of mammals (Berntson and Micco, 1976; Rose, 1990) and nonmammalian vertebrates alike (Ewert, 1987; Rose, 2000).

In fishes, the degree to which most aspects of neurobehavioral function are controlled by the brainstem and spinal cord is extreme, as shown by experiments in which the cerebral hemispheres have been removed from diverse species of fishes, leaving only the brainstem and spinal cord intact (Overmier and Hollis, 1983). The behavior of these fishes is strikingly preserved. They still find and consume food, show basic capabilities for sensory discrimination (except for the loss of the sense of smell, which is processed entirely in the forebrain) and many aspects of social behavior, including schooling, spawning, and intraspecies aggression. Although there are some species differences, courtship, nest building, and parental care often persist after forebrain removal. Most of the forms of learning of which fishes are capable are intact in the absence of the forebrain, although avoidance learning seems to be much more difficult for fish with the cerebral hemispheres removed (Overmier and Papini, 1986). This difficulty with avoidance learning is not due to reduced responsiveness to noxious stimuli because the reflexive and locomotor, including escape, responses to such stimuli by fish without cerebral hemispheres appear to be quite normal. The general conclusion that emerges from many studies is that the basic patterns of fish behavior are controlled by lower brain structures, mainly the brainstem and spinal cord. The cerebral hemispheres serve mainly to “modulate” behavior, that is, to regulate its intensity or frequency and to refine its expression (Overmier and Hollis, 1983). Thus, the neurobehavioral evolution of fishes has resulted in a highly diversified array of species in which the essentials of neurobehavioral function are mediated mainly by neural systems below the cerebral hemispheres.

Two other lines of evidence demonstrate that the brainstem rather than the forebrain is the dominant level of processing and neurobehavioral control. First, implicit learning occurs without conscious awareness, even in humans (Kolb and Whishaw, 1995; Macphail, 1998), so the presence of this type of learning in fishes does not constitute a capacity for awareness. Unlike the subsequent course of mammalian emergence, the evolution of fishes placed them in a niche where neurobehavioral processes that could be mediated by a brain comprised mainly of brainstem structures and relatively small cerebral hemispheres was adequate for their fitness needs. Fishes, being a highly diverse taxon, have extreme variations in brain structure, especially at the brainstem level (Nieuwenhuys *et al.*, 1998b; Butler and Hodos, 1996). It is important to emphasize that fish brains can have highly complex, unique adaptations due to their diverse evolutionary paths. Consequently, these brains are not merely simpler versions of mammalian (or amphibian or reptilian) brains (Nieuwenhuys *et al.*, 1998b; Butler and Hodos, 1996; Wulliman, 1998).

In spite of the diversity and complexity among species, the behaviors of fishes are nonetheless highly stereotyped and invariant for a given species. Such stereotyped species-typical behaviors, however, are not simple or merely reflexive. Species-typical behaviors in vertebrates are known to be controlled by motor patterning mechanisms that are far more complex than reflexes (Ewert, 1987; Fentress, 1987). The basic behaviors involved in reproduction, feeding and drinking, escape or defense, and reactions to noxious stimuli, are controlled by motor patterning mechanisms that are located mainly in the brainstem and spinal cord of mammals (Berntson and Micco, 1976; Rose, 1990) and nonmammalian vertebrates alike (Ewert, 1987; Rose, 2000). In fishes, the degree to which most aspects of neurobehavioral function are controlled by the brainstem and spinal cord is extreme, as shown by experiments in which the cerebral hemispheres have been removed from diverse species of fishes, leaving only the brainstem and spinal cord intact (Overmier and Hollis, 1983). The behavior of these fishes is strikingly preserved. They still find and consume food, show basic capabilities for sensory discrimination (except for the loss of the sense of smell, which is processed entirely in the forebrain) and many aspects of social behavior, including schooling, spawning, and intraspecies aggression. Although there are some species differences, courtship, nest building, and parental care often persist after forebrain removal. Most of the forms of learning of which fishes are capable are intact in the absence of the forebrain, although avoidance learning seems to be much more difficult for fish with the cerebral hemispheres removed (Overmier and Papini, 1986). This difficulty with avoidance learning is not due to reduced responsiveness to

noxious stimuli because the reflexive and locomotor, including escape, responses to such stimuli by fish without cerebral hemispheres appear to be quite normal. The general conclusion that emerges from many studies is that the basic patterns of fish behavior are controlled by lower brain structures, mainly the brainstem and spinal cord. The cerebral hemispheres serve mainly to "modulate" behavior, that is, to regulate its intensity or frequency and to refine its expression (Overmier and Hollis, 1983). Thus, the neurobehavioral evolution of fishes has resulted in a highly diversified array of species in which the essentials of neurobehavioral function are mediated mainly by neural systems below the cerebral hemispheres. Two other lines of evidence demonstrate that the brainstem rather than the forebrain is the dominant level of processing and neurobehavioral control. First, the cerebral hemispheres of fishes tend to be smaller, in comparison with the brainstem, than in mammals (Nieuwenhuys *et al.,* 1998a). This is important because size of a brain region is typically related to its functional significance. Second, extreme behavioral specialization in fishes is associated with expanded brainstem as opposed to cerebral hemisphere development (Figure 1). For example, electric rays have an enormously developed electromotor lobe on the dorsal medulla. Neurons in this lobe innervate the electric organ in the pectoral disc, which the rays use for defense as well as for stunning prey. In some rays, this lobe alone comprises 60% of the total brain weight (Nieuwenhuys *et al*., 1998b). Similarly, chemosensory behavioral specialization is associated with large lobes on the dorsal medulla that process sensory input from taste receptors (Nieuwenhuys *et al*., 1998a; Sorensen and Caprio, 1998). The cerebral levels of this sensory pathway for taste are much smaller than the brainstem level, constituting what is known as a diminishing system (Nieuwenhuys *et al*., 1998b). In contrast to these extremely specialized fish species, are many species regarded as more generalized, such as hexanchiform sharks and dipnoans, which have slender, elongated brainstems, without any enlarged subsystems (Nieuwenhuys *et al*., 1998a). In comparison with the prominence of the brainstem, the cerebral hemispheres of fishes are comparatively small, even diminutive, especially if the purely olfactory regions of the cerebrum are excluded (Figure 1). There are, however, rather great variations between the agnathans, chondrichthyans, and osteichthyans in the degree of cerebral hemisphere development, with relative size and differentiation of the cerebrum tending to increase somewhat across these three groups, respectively (Nieuwenhuys *et al*., 1998a).

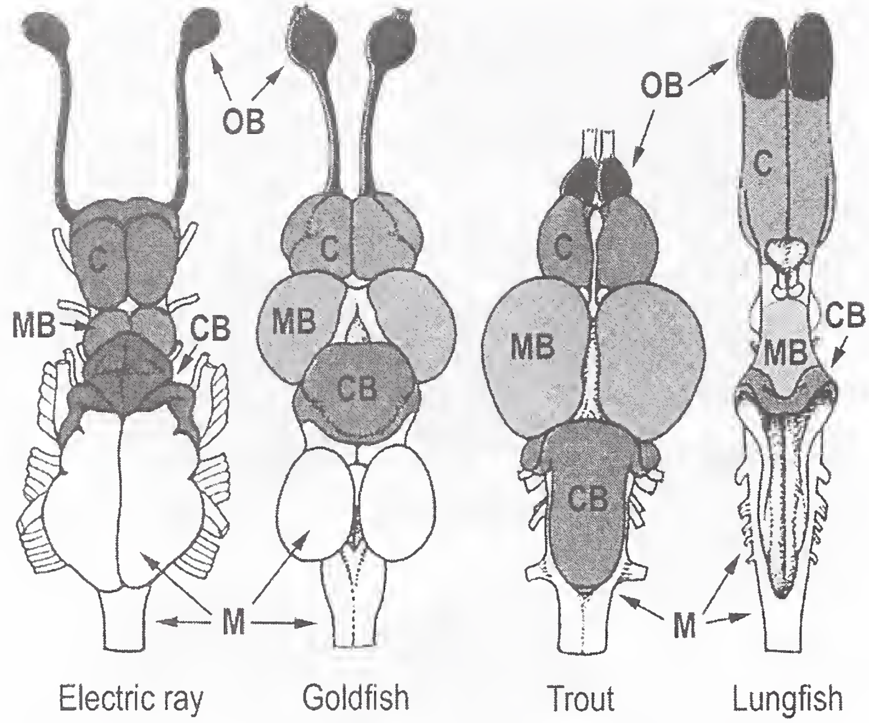


FIGURE 1. Comparison of brain structure in four fish species. These dorsal views show the basic similarity of brain structural organization in diverse types of fishes, yet certain striking differences related to function are clearly apparent. Structural specializations are most pronounced in the brainstem, which consists of the medulla, cerebellum and midbrain. The electric ray (Raja clavata) has a large electromotor nucleus on the dorsal surface of the medulla (shown by the upper arrow pointing to the medulla). The goldfish (Carassius auratus) has a large vagal lobe (upper arrow pointing to the medulla) due to its extensively developed chemosensory system for taste. The rainbow trout (*Oncorhynchus mykiss*) has a relatively large optic tectum of the midbrain due to its visual specialization. The South American lungfish (Neoceradotus forsteri), regarded as an unspecialized species, has a slender brain lacking structural exaggerations. Redrawn from Nieuwenhuys et al., 1998a. Abbreviations: C — cerebral hemisphere; CB — cerebellum; M — medulla; MB — midbrain (the optic tectum is the only midbrain structure visible in this dorsal view); OB — olfactory bulb.

VI. THE MAMMALIAN RADIATION IS ASSOCIATED WITH CEREBRAL HEMISPHERE EXPANSION AND GREATER CEREBRAL HEMISPHERE DOMINANCE OVER NEUROBEHAVIORAL FUNCTION

Mammalian evolution, similar to that of fishes, has exhibited increasing diversification and complexity. Unlike the brainstem-based specializations in fishes, however, behaviorally specialized mammals have conspicuously developed cortical regions. Neural systems that are enlarged at the cerebral hemisphere level, such as the complex visual cortex of the owl monkey (Allman, 1999), are known as expanding systems and are characteristic of mammals (Nieuwenhuys *et al.*, 1998b). Of particular significance in mammals is the development of a unique, six-layered type of cerebral cortex called neocortex. The development and expansion of this cortex has embellished sensory processing and motor control greatly. In addition, as discussed earlier, the massively expanded neocortex in humans appears to be the foundation of our consciousness.

Mammals, similar to fishes, are highly diversified. They include specialists as well as generalists, with considerable neurological diversity at the level of the cerebral hemispheres. Humans represent an extreme case of cerebral hemisphere and neocortical expansion. The volume of the human

cerebral cortex is 3.2 times larger than that of a chimpanzee of similar body weight, whereas the size of the lower brainstem is nearly the same in the two species (Deacon, 1992a; Kolb and Whishaw, 1995). This massive neocortical expansion has resulted in several transformations of the human brain that are qualitative rather than just quantitative. These transformations include a large prefrontal cortical region that mediates long-term planning and language-specialized regions of the frontal, temporal, and parietal lobes. The expansion of the cerebral hemispheres has also allowed lateralized functions of the two cerebral hemispheres, such that the left hemisphere has a predominant role in language and the right hemisphere in visual-spatial processing, both of which are manifestations of higher-order consciousness (Kolb and Whishaw, 1995).

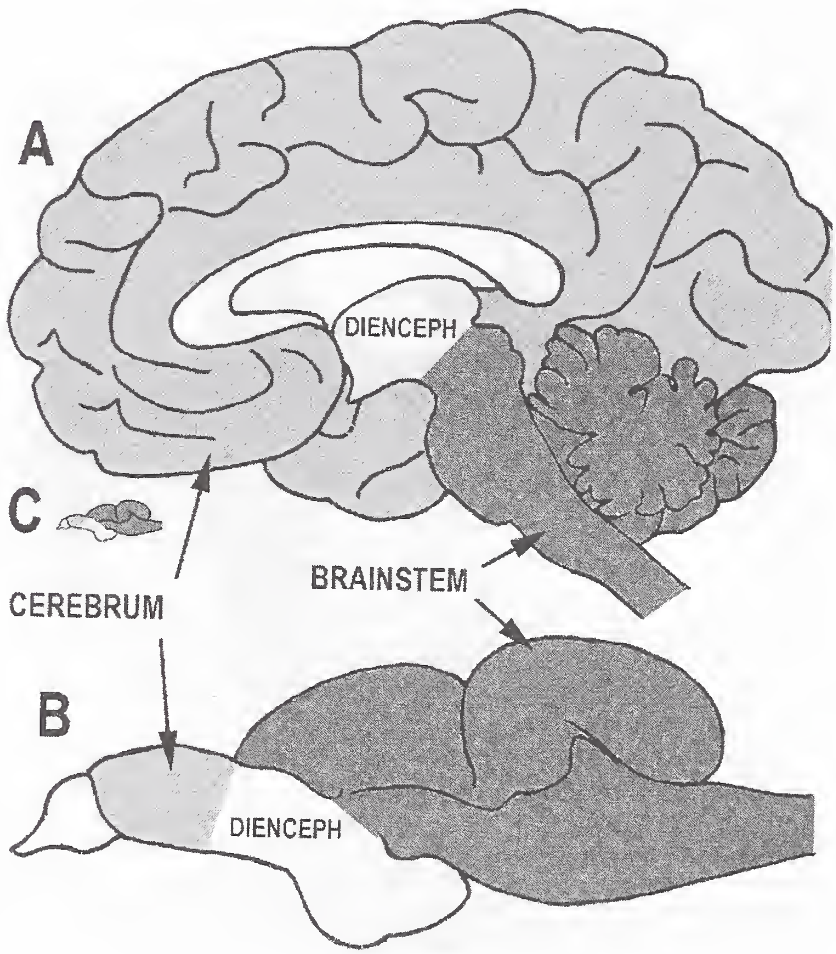
The expansion of the cerebral hemispheres in mammals has resulted in a greater dependency on this brain level, especially in humans. Cerebral cortex destruction leaves a human in a persistent vegetative state in which all conscious awareness is abolished (Figure 3). Sleep-wake cycles and reactions to noxious stimuli persist in such cases due to mediation of these processes by lower levels of the central nervous system (Jouvet, 1969; Young *et al.,* 1998). Although many of the more stereotyped behaviors of humans and other mammals are generated by brainstem and spinal systems, these behaviors are still very dependent on support by the cerebral hemispheres for effective functioning (Berntson and Micco, 1976; Grill and Norgren, 1978; Huston and Borbely, 1974). Non-primate mammals are capable of a greater range of functional behavior after the destruction of the cerebral hemispheres. These animals exhibit locomotion, postural orientation, elements of mating behavior, and fully developed behavioral reactions to noxious stimuli (Berntson and Micco, 1976; Rose, 1990; Rose and Flynn, 1993). However, unlike fish with similar brain damage, these behaviors are not really functional and such mammals cannot survive without supportive care, including assisted feeding. Thus, the brainstem-spinal system in mammals contains the neural circuitry for basic, stereotyped behavioral programs, as it does in nonmammalian vertebrates such as fishes, but mammals have greater dependence on the cerebral hemispheres for functionally effective behaviors.

VII. THE SIGNIFICANCE OF NEOCORTICAL EVOLUTION – THE QUESTION OF CONSCIOUS AWARENESS.

As stated previously, the neural substrates necessary for consciousness are becoming more clearly defined. Although consciousness cannot be explained in detail, we know when it is present or absent and we know what parts of the human brain are required to have it. In contrast to the diverse and extensive evidence that consciousness depends on neocortical functioning, some writers, most notably Donald Griffin (1976, 1992), have argued for the existence of conscious awareness in diverse, nonmammalian vertebrates and invertebrates. A major problem with this proposal is that it has been made without consideration of its feasibility. Specifically, the nervous systems of invertebrates not only lack any type of cortex, but they also are organized in fundamentally different ways, usually with far fewer neurons than a mammalian brain. Likewise, the cerebral hemispheres of fishes have a more rudimentary structure that differs substantially from the structure of mammalian neocortex. A simple type of three-layered, “general cortex” is present in the cerebral hemispheres of reptiles, but true neocortex, with its greatly enriched information processing capacity is found only in mammals (Nieuwenhuys, 1998a; Northcutt and Kaas, 1995).

Whereas the qualitative differences in cortical structure and function are quite pronounced between reptiles and the simplest mammals, these differences are overshadowed by the large differences in neocortical structure and function found between orders of mammals. The proportion of the mammalian brain that is neocortex varies greatly. Insectivores such as hedgehogs, which are thought to resemble the early mammals, have fairly small amounts of neocortex. Simians have45.5 times more neocortex for a given body size than primitive insectivores and humans have 145 times more neocortex than the most primitive mammals (Stephan and Andy, 1964). This enlargement of human neocortex is accompanied by greater cortical structural differentiation and functional diversification (Mountcastle, 1998).

FIGURE 2. Comparison of human brain with a trout brain. (A) Diagram of a midline view of a human brain. The cerebral hemisphere, comprised mostly of neocortex, is light gray and the brainstem is dark gray. The diencephalon (DIENCEPH) consists of the thalamus (dorsal half), which is connected with the cortex of the cerebral hemispheres and the hypothalamus (ventral half), which is connected mainly to subcortical structures. (B) Diagram of a side view of a rainbow trout brain. The cerebral hemisphere is very small relative to the size of the brainstem. The diencephalon of the trout is mostly hypothalamus. The white structure at the left of the cerebral hemisphere is the olfactory bulb. (C) Brain of a 30-cm rainbow trout shown at the same scale as the human brain diagram.



The fact that all forms of human consciousness require neocortical functioning leads to two possible conclusions: (1) non-mammals simply cannot have consciousness, even primary consciousness, because they lack the known neural requirement for it, or (2) these organisms are able to generate consciousness by a different neurological process. This issue is examined below in several ways.

Because it is known that neocortex is necessary for consciousness in humans, it might also be assumed that other animals with neocortex, that is all mammals, should have some form of consciousness as well. In practice, there is a wide range of beliefs or working assumptions about this matter among neuroscientists. Macphail (1998) has argued that evidence from behavior warrants the conclusion that nonhuman mammals cannot have consciousness of any type. In contrast, some neuroscientists routinely use primates to investigate the cortical neural mechanisms

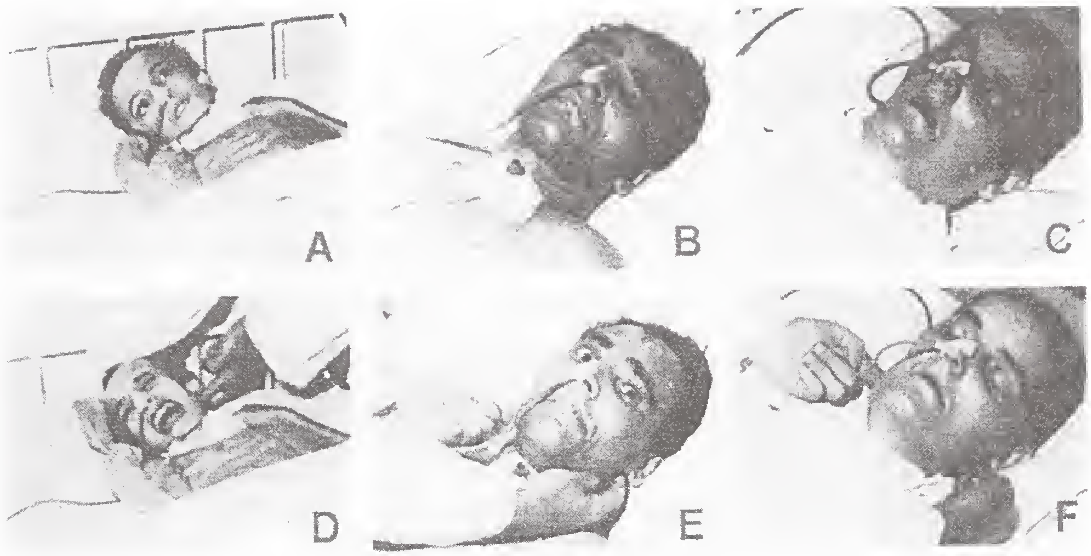


FIGURE 3. Behavioral responses to noxious stimuli in people with decortication syndrome and unconsciousness. A — A decorticate individual with wakefulness but no consciousness. Noxious stimulation consisting of pressure on the mastoid processes of the skull (D) evokes a facial grimace and cry, similar in appearance to those likely to occur in a normal, conscious individual. Such decorticate individuals may also push at the hands of the examiner. B — A decorticate, unconscious individual who is not spontaneously awake but shows waking and a facial display (E) during noxious stimulation. C — An individual with brainstem damage who is unconscious and shows no waking or facial display during noxious stimulation (F). These individuals are still capable of spinally mediated withdrawal responses to noxious stimulation of a limb (Jouvet, 1969). (Reprinted with the author's permission.)

underlying primary consciousness (Edelman and Tononi, 2000; Koch and Crick, 1999, 2000). While many neuroscientists seem to assume the existence of primary consciousness in at least some mammals, particularly primates, extended consciousness is generally considered a uniquely human capacity (Donald, 1991; Edelman and Tononi, 2000).

A critical point in this analysis is the fact that a large part of the activity occurring in our brain is unavailable to our conscious awareness (Dolan, 2000; Edelman and Tononi, 2000; Koch and Crick, 2000; Libet, 1999; Merikel and Daneman, 2000). This is true of some types of cortical activity and is true for all brainstem and spinal cord activity. We are unaware of activity confined to primary sensory cortex (Koch and Crick, 2000; Lamme and Roelfsma, 2000; Laureys *et al.*, 2000c; Libet, 1999; Rees *et al.*, 2000). We also have no conscious contact with the massive numbers of neurons in our cerebellum, despite the fact that these neurons are intensely active, controlling many aspects of movement and posture. Likewise, we are unaware of the activity of neurons in our hypothalamus, whose firing regulates our heart rate, blood pressure, and neuroendocrine function. Thus, for organisms such as fishes, which have no neocortex at all, it seems entirely logical that none of their brain activity could be consciously experienced. Although computer analogies with brain function are often misleading, a simple example may help to communicate this argument. Consciousness functions as a monitor that gives us awareness of some but not all operations that are occurring in our brain. In our consciousness, most of the activity in the spinal cord, brainstem, and some parts of the cortex is not displayed on the monitor of our consciousness, so we are unaware of it despite its effective functioning. In animals without the consciousness monitor provided by the neocortex, brain and spinal cord activity function effectively, as they do in our subcortical systems, without any means for reaching awareness or any need for it, just as programs do in a computer with the monitor off.

VIII. THE NEUROPSYCHOLOGICAL BASIS OF PAIN IN HUMANS

A. PAIN IS A PSYCHOLOGICAL EXPERIENCE THAT IS SEPARATE FROM BEHAVIORAL REACTIONS TO INJURIOUS STIMULI

Extensive empirical research on the human pain experience has provided a definition of pain formulated by the International Association for the Study of Pain (Wall, 1999): (1) pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage; (2) pain is always subjective; (3) pain is sometimes reported in the absence of tissue damage and the definition of pain should avoid tying pain to an external eliciting stimulus. One of the most critical concepts about pain is the distinction between nociception and pain. As Wall (1999) emphasized, nociception, which is "…activity induced in the nociceptor and nociceptive pathways by a noxious stimulus is not pain, which is always a psychological state." The points critical to understanding differences between fishes and humans with respect to pain are that: (1) pain is both a sensory and emotional experience (that requires conscious awareness) and (2) nociception does not result in pain unless the neural activity associated with it reaches consciousness.

A commonplace experience helps to illustrate the distinction between nociception and pain in humans. When a dentist injects a local anesthetic to block conduction in part of the trigeminal nerve, surgery on a tooth still excites nociceptive receptors that normally trigger pain. However, the nerve block, being administered between the site of surgery and the nerve's connection with the brain, prevents this activity from reaching the brain. Thus, there is no behavioral reaction to the nociceptive stimulus and no pain. Another informative example is that of a spinal injury that transects the cord in a human. A noxious stimulus applied to a limb below the level of the spinal transection excites nociceptive sensory receptors and nociceptive pathways within the spinal cord. This nociceptive activity at the spinal level produces a limb withdrawal response, but because nociceptive pathways are interrupted between the spinal cord and the neocortex, no pain is felt.

B. NOCICEPTIVE REACTIONS IN ANIMALS

The distinction between nociception and pain is critical to the question of whether fishes can experience pain because nociception-based behaviors are commonly confused with the pain experience. The capacity to react to injurious or threatening stimuli is a universal characteristic of animal life. Thus, the presence of reactions to injurious stimuli in unicellular forms that have no nervous systems and in primitive invertebrates that have no brains demonstrates that reactivity to noxious stimulation, per se, can occur in the absence of awareness of such stimuli (Dewsbury and Rethlingshafer, 1973; Bullock *et al.*, 1977). In all vertebrates, including humans, reactions to injurious stimuli are generated by neural systems in the spinal cord and brainstem. Vertebrates generally have more complex nervous systems than invertebrates and, unlike most invertebrates, they have a clearly developed brain. This brain receives information from the spinal cord and cranial nerves about noxious stimuli that contact the body surface and head, respectively. Working together with the spinal cord, the brain generates responses that cause the organism to "escape" or "avoid" these stimuli. These responses are produced by innate neural programs and include withdrawal of the stimulated body part, struggling, locomotion, and in some animals vocalizations. All of these responses are generated by lower levels of the central nervous system: the brainstem and spinal cord. This is known because animals with the cerebral hemispheres removed leaving the brainstem and spinal cord intact are fully capable of exhibiting the typical "pain-like" behavioral reactions to injurious stimuli (Berntson and Micco, 1976). As explained below, this same functional scheme for generation of nociceptive responses applies to humans.

C. A SYNOPSIS OF THE NEUROLOGICAL BASIS OF PAIN IN HUMANS

The following is a brief summary of what is known about the neural basis of pain in humans. Primary literature references for this account are available in a recent review of this subject by Price (1999) and a more comprehensive volume by Wall and Melzack (1994). The human pain experience, as explained previously, is a psychological process of the brain, separate from the behavioral responses to nociceptive stimuli. It is a complex, multifaceted experience with at least three, intertwined dimensions (Price, 1999; Melzack and Fuchs, 1999): (1) a sensory informational component that conveys the locus of noxious stimulation, its physical features, and its intensity; (2) an emotional dimension that constitutes the suffering and unpleasantness of the experience; and (3) a cognitive-evaluative component involving attention, previous experience and the perceived threat to the individual. All of these facets of the pain experience are predicated on concurrent consciousness for their existence; an unconscious person would experience none of these aspects of pain.

Noxious stimuli activate sensory receptors called free nerve endings (Figure 4). These receptors consist of two varieties, a type that responds specifically to injurious stimuli (nociceptors) and a type that responds to other somatic stimuli, but especially to intense noxious stimuli with greatest firing rates (polymodal nociceptors). It should be emphasized that it is incorrect to characterize nociceptors as "pain receptors" because activation of these receptors initiates only nociceptive neural activity, which, by itself, is inadequate for producing the psychological experience of pain. Activity is transmitted to the spinal cord from free nerve endings by two types of nerve fibers, small-diameter myelinated axons and small-diameter unmyelinated axons. A similar system exists for nociception involving the face and mouth through the trigeminal nerve and its brainstem connections. The myelinated axons are principally responsible for eliciting "first pain", such as that from a pinprick, which is rapidly experienced, of short duration, well localized and not especially unpleasant. The unmyelinated axons are responsible for eliciting "second pain", which is slowly experienced, poorly localized, long-lasting and very unpleasant. Both types of axons synapse in the dorsal gray matter of the spinal cord, where subsequent processing of their input signals occurs in diverse types of neurons. Spinal cord neurons send axons to the brain, mainly through the ventrolateral spinal tract. This pathway is also called the spinothalamic tract because many of its component axons travel all the way to the thalamus in the brain before synapsing. Of additional importance, however, is a complex network of neurons in the reticular formation of the brainstem that also receives connections from the spinothalamic tract. This reticular network processes nociceptive information and transmits it to diverse subcortical brain structures, including parts of the thalamus. In addition, this brainstem reticular network generates innate behavioral responses to nociceptive stimuli. Our fundamental behavioral reactions to noxious stimuli, including vocalization, facial grimacing, and withdrawal, are mediated by subcortical brain and spinal systems (Jouvet, 1969; Kandel *et al.*, 2000; Laureys *et al.*, 1999, 2000a,b; Young *et al.,* 1998). Activation of these responses by noxious stimuli can occur without consciousness in people with extensive cortical damage (Figure 3) and in humans born without cerebral hemispheres (Kolb and Whishaw, 1996;

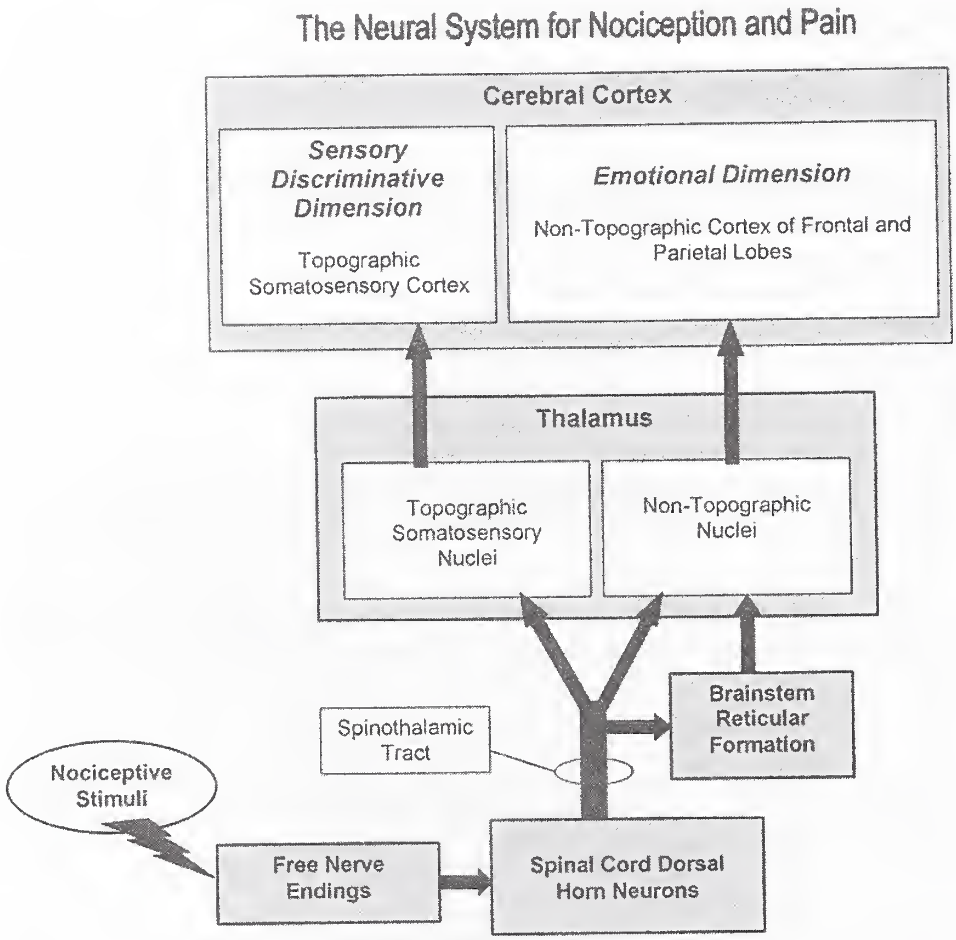


FIGURE 4. The ascending neural system for nociception and pain in humans. Nociceptive stimuli activate free nerve endings in body tissues. This activity is conducted the dorsal spinal gray matter (dorsal horn) through small-diameter unmyelinated and small-diameter myelinated nerve fibers. Spinal neurons project to the brain, through the spinothalamic tract, to two somewhat separate systems. One system encodes locations of stimuli on the body (topographic organization) and provides a sensorydiscriminative dimension to pain at the somatic sensory cortical level. In this system, the spinothalamic tract connects directly to topographic somatosensory nuclei of the thalamus, which project to the topographic, primary somatosensory cortex. Another system receives projections from the spinothalamic tract at two levels, one division going directly to non-topographic nuclei in the thalamus and another connecting with neurons of the brainstem reticular formation. Some of the latter neurons project to nontopographic thalamic nuclei. Nociception-related activity from the nontopographic thalamic nuclei is projected to diverse nontopographic regions of the cortex, principally in the frontal lobes. Activity in these cortical regions is responsible for the emotional unpleasantness (suffering) dimension of pain. For greater clarity, the descending components that regulate ascending nociceptive activity have not been included in this diagram. (See the text for additional explanation.)

Steiner, 1987). Thus, the behavioral displays related to noxious stimuli or emotion in humans, as in other animals, are stereotyped, automatic behavioral programs controlled by lower levels of the central nervous system, and these responses can be evoked without any corresponding awareness of noxious stimuli. Limb withdrawal and leg locomotor responses, of course, are produced directly at the spinal cord level.

The direct spinothalamic tract terminates, in large measure, in a somatotopically organized region of the thalamus that processes diverse types of body sense information and projects to the primary somatic sensory cortex. This primary cortex also is organized somatotopically for analyzing locations of body stimuli. A separate part of the spinothalamic tract, as well as nociceptive signals transmitted through the brainstem reticular network, converge on nontopographically organized nuclei of the thalamus. These thalamic nuclei project to multiple cortical regions, mainly in the frontal and parietal lobes. Thus, there are two major routes through which nociceptive activity reaches the cortex: one that preserves spatial information about the stimulus and another that influences a much wider cortical territory, but does not encode spatial information.

Several caveats are required in this highly simplified presentation. First, the multiple ascending nociceptive pathways that generate pain experience at the cortical level work in concert. Consequently, there is no specific structure that can be designated a "pain pathway", "pain nucleus" or "cortical pain zone". Second, there is a "gate control" system in the dorsal gray matter of the spinal cord that actively suppresses the transmission of nociceptive activity to the brain. In addition, there is a system descending from the brainstem that contains opioid neuropeptides and suppresses the upward flow of nociceptive activity. Thus, there are spinal and brainstem systems that can powerfully attenuate the transmission of nociceptive information to the higher levels of the brain, and, ultimately, the cortical level of pain perception.

Probably the greatest advance in our knowledge about pain in recent years is understanding the role of the cortex. This information is of particular importance to a consideration of whether fishes can experience pain. A variety of evidence shows that diverse frontal and parietal lobe cortical regions are involved in generating the conscious experience of pain (Figure 5). The sensory properties of nociceptive stimuli, including location, stimulus characteristics (burning, crushing, piercing) and spatial extent, are signaled by activity in the somatotopically organized somatosensory cortex area of the parietal lobe. In contrast, pain intensity is signaled by activity in more widespread areas of the frontal and parietal lobes, involving all of the regions that are implicated in cortical processing of pain. The emotional unpleasantness of pain is processed predominantly by frontal lobe cortex, including the anterior cingulate gyrus, the insula, and prefrontal cortex. In brain imaging studies based on magnetic resonance imaging and positron emission tomography, the former two structures have shown activity specifically associated with the perceived unpleasantness of pain (Coghill and Duncan, 1999; Ploghaus *et al.*, 1999; Rainville *et al.*, 1997; Xu *et al.*, 1997). The anterior cingulate gyrus is thought to be especially important for processing the emotional unpleasantness of pain. It is a unique type of fivelayered cortex, known as mesocortex, nearly identical in structure with neocortex and specific to mammals, but also having unique structural features in great apes and humans (Nimchinsky *et al.*, 1997). For simplification of this discussion, cingulated cortex will be included in references to neocortex.

Independent confirmation of the importance of frontal lobe structures for the perception of the unpleasantness of pain comes from a long history of neurosurgical procedures. Many people have undergone a destruction of a portion of the anterior cingulate gyrus to alleviate severe, chronic pain (Bouckoms, 1994; Cosgrove and Rausch, 1998; Tasker, 1994). Persons receiving such surgery report that the pain is still present (the sensory-informational aspect of the pain), but it is no longer unpleasant or distressing (the emotional suffering aspect of the pain). A similar effect on pain experience was found to accompany prefrontal lobotomies (Bouckoms, 1994). After such prefrontal cortex damage, the tendency to perceive pain as unpleasant and something that is a cause for

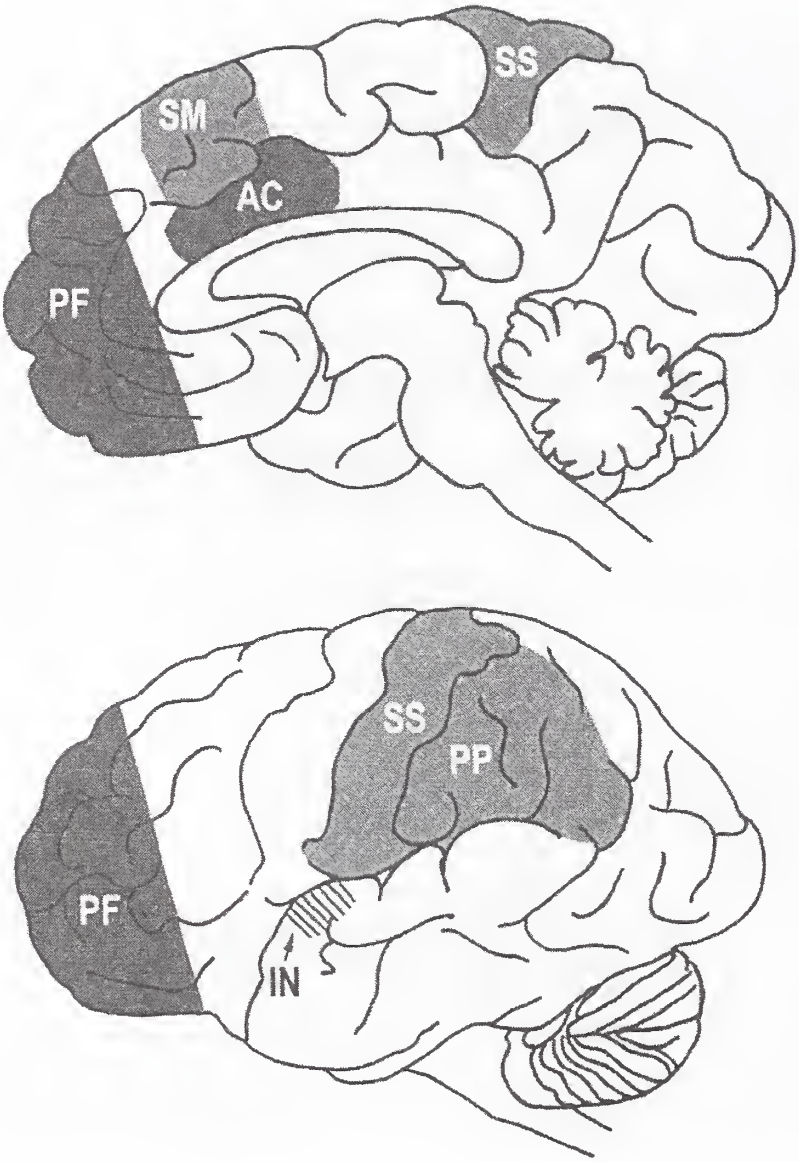


FIGURE 5. Cortical regions involved in the human pain experience. These regions have been identified by magnetic resonance imaging, positron emission tomography, neurosurgical procedures, neurological case studies, and electrophysiological recording (Price, 1999; Treede et al., 1999). AC — anterior cingulate gyrus; IN — anterior insula, a frontal cortical region covered by the temporal lobe; PF — prefrontal cortex; SM — supplemental motor cortex; SS — primary somatic sensory cortex of the postcentral gyrus and the small, adjacent second somatic sensory area (not labeled). PP — posterior parietal cortex. SS is critical for the sensory-discriminative dimension of pain. The intensity dimension of pain is perceived by activation of multiple regions, including AC, IN, PP, SM, and SS. Low-intensity stimuli activate cortex of the contralateral hemisphere, while more intense stimuli activate these cortical regions in both hemispheres. The cognitive-evaluative component of pain, involving attention to the pain, perception of a threat to the individual and conscious initiation of strategies for dealing with the pain depends on AC, PF, and SM.

concern is greatly diminished. The effects of these neurosurgical procedures are one line of evidence showing that the cognitive-evaluative components of pain (attention to the pain, perceived threat to the individual, and conscious generation of strategies for dealing with the pain), are based on frontal lobe structures, especially the prefrontal cortex and anterior cingulate gyrus. There may be other cortical regions and processes that are important for the totality of the pain experience. The most important point here is that the absolute dependence of pain experience on neocortical functions is now well established (Price, 1999; Treede *et al.*, 1999).

It is also revealing to note that the cortical regions responsible for the experience of pain are essentially the same as the regions most vital for consciousness. Functional imaging studies of people in a persistent vegetative state due to massive cortical dysfunction (Laureys *et al.*, 1999, 2000a,b) showed that unconsciousness resulted from a loss of brain activity in widespread cortical regions, but most specifically the frontal lobe, especially the cingulate gyrus, and parietal lobe cortex. In these unconscious individuals, nociceptive stimulation caused strong activation of the brainstem and thalamus, and in some cases the primary somatic sensory cortex. However, in the absence of more widespread cortical function, this nociceptive stimulation did not generate consciously perceived pain. Recall, as explained earlier, that individuals in this functionally decorticate condition can still make organized motor responses to nociceptive stimuli and appear to be wakeful (Figure 3). Thus, intense nociceptive activation of subcortical structures, and even limited cortical regions, is inadequate for conscious awareness of pain.

IX. THE QUESTION OF PAIN AND DISTRESS IN FISHES

A principal cause for assumptions that fishes feel pain is an anthropocentric interpretation of reactions by a fish to stimuli that probably would cause pain in a human. There is no scientifically valid evidence in support of this assumption. Compared with mammals, however, fishes have received much less investigation of nociceptive function. Nevertheless, neuroanatomical, neurophysiological, and behavioral findings from studies of elasmobranchs lead to serious doubt that sharks or rays have an adequate neural system even for nociception. First, none of several elasmobranch species that have been studied had many unmyelinated sensory axons in nerves entering the spinal cord (Coggeshall *et al.*, 1978; Snow *et al.*, 1993). This is critical because unmyelinated nerve fibers are the predominant type responsible for signaling the occurrence of nociceptive stimuli and tissue injury (Price, 1999; Wall, 1999). Humans and other mammals have numbers of unmyelinated spinal nerve fibers have insensivity to pain (Willis, 1985). Second, the first layer in the spinal dorsal gray matter, a region where nociception-signaling sensory fibers normally synapse, was not present in any of four species of sharks and rays investigated (Cameron *et al.*, 1990). Third, a neurophysiological study in a stingray revealed an absence of typical nociceptive sensory neurons innervating body tissues (Leonard, 1985). Lastly, observations of sharks have documented that severe lacerations and wounds did not interfere with feeding behavior (Snow *et al.*, 1993). Collectively, these observations with diverse species lead to the conclusion that elasmobranchs lack the neural structures for processing nociceptive information, much less sensing pain.

A lack of nociceptive responsiveness may constitute something of an advantage to elasmobranchs feeding on otherwise noxious prey. For example, individual specimens of the great hammerhead, *Sphyrna mokarran*, which preys on stingrays, have been found with numerous (as many as 96) stingray barbs embedded in the mouth, throat, and tongue (Helfman *et al.,* 1997). Another important point is that sharks and rays, as well as other fishes, react with “escape” responses to being caught by hook and line angling in spite of their apparent deficiency in detecting nociceptive stimuli. This fact suggests, as has been concluded from some observations of teleosts (Gregory, 1999), that more unmyelinated than myelinated spinal sensory nerve fibers and humans with greatly reduced

interference with their free movement is a major factor creating flight responses rather than noxious stimulation from a hook. Of course, diverse taxa of fishes eat prey having spines, sharp fin rays, or thorny exoskeletons. It would seem adaptive for such fishes to have somewhat low nociceptive reactivity, especially in the oral cavity. It would also seem logical for there to be sufficient reactivity to prevent excessive trauma to the predator, although this logic does not appear to apply well to elasmobranchs.

Neuroanatomical and neurophysiological studies of nociceptive sensory nerve fibers similar to those in sharks and rays apparently have not been done in teleost fishes. However, anatomical studies have identified free nerve endings in the skin tissues of teleosts (Whitear, 1971). As explained above, free nerve endings are sensory receptors that transduce diverse types of somatic stimuli, including nociceptive ones. Although it remains to be demonstrated that in teleosts these receptors actually respond differentially to nociceptive stimuli, their presence suggests that bony fishes, unlike elasmobranchs, may have receptors for detection of nociceptive stimuli.

In spite of the differences between elasmobranchs and teleosts in peripheral nerve and spinal gray matter components of nociceptive processing, neuroanatomical studies have shown that the fundamental spinal and brainstem pathways for somatic sensory transmission are present in both elasmobranchs (Ebbeson and Hodde, 1981) and teleosts (Oka *et al.*, 1986; Wulliman, 1998). These pathways constitute a dorsal spinal tract that terminates in the lower brainstem and a lateral spinal pathway that terminates at multiple brainstem levels (Oka *et al.,* 1986). There is remarkable variation among teleost species in the exact structures and levels of brainstem termination of the ascending lateral spinal pathway (Wulliman, 1998). Unlike the somewhat clearer interpretations that can be made from studies of unmyelinated, peripheral sensory fibers, the function of these spinal cord-brainstem pathways is less clear from purely anatomical data. To generalize from mammals, the lateral ascending spinal pathway in fishes, comparable to the spinothalamic tract of mammals, would convey diverse somatic sensory information, but would also be the pathway with ascending connections of most importance for nociception (Wall, 1999; Price, 1999). One interpretation stemming from these neuroanatomical data is that the basic spinal cord and brainstem structures that process nociceptive stimuli by mammals are present (albeit with more variation) in fishes. This interpretation must be qualified by the fact that the same spinal-brainstem pathway is present in sharks, which probably lack a capacity for nociception. Thus, the anatomical presence of an ascending lateral spinal-brainstem system is not evidence for a nociceptive function as opposed to purely mechanosensory function. Of course, for conscious pain experience, as opposed to just nociception, a large amount of neocortex appears to be required. As discussed below, there is no such structure in brains of fishes.

Functional evidence of nociception in fishes comes from studies in which fish were trained with an electric shock stimulus in either Pavlovian “fear” conditioning or instrumental avoidance conditioning paradigms (Davis and Klinger, 1994; Overmier and Hollis, 1983). In such studies, an electric shock of sufficient intensity to cause behavioral reactions can be used to produce behavioral avoidance responses or classically conditioned responses such as changes in branchial movements. It should be reiterated that these conditioning tasks are examples of implicit learning, which, as stated earlier, occurs without conscious awareness. Whether an electrical shock delivered to a fish immersed in water has the same nociceptive properties as an electric shock applied to the skin of humans is unknown. However, findings showing that morphine can reduce reactivity to electric shocks in goldfish, and that opiate antagonists such as naloxone reduce the effects of morphine (Ehrensing *et al.* 1982), tend to support the notion that electric shocks given to a fish activate some type of nociceptive process. However, as explained elsewhere, the nociceptive reactions of a fish to electric shocks do not, by themselves, demonstrate “pain” experience, which requires conscious awareness. Furthermore, opiates, such as morphine, are known to produce their antinociceptive effects by acting at subcortical brainstem and spinal cord sites (Price, 1999). Thus, opiate effects on nociceptive processing are not evidence of a capacity for consciously perceived pain. There is

also a major problem in referring to these classic conditioning paradigms with fish as "fear" conditioning. As explained below, awareness of emotions, such as fear, is in a similar neuropsychological category as awareness of pain; it requires participation of cortical brain systems that fishes do not have.

A. IS THERE A NON-NEOCORTICAL MECHANISM FOR GENERATING CONSCIOUSNESS IN FISHES?

Neocortex, the essential brain level for conscious awareness of pain, does not exist in the brains of fishes. However, it might be argued that conscious awareness of experiences exists in fishes through some mechanism other than the neocortically based consciousness of humans (Verheijen and Flight, 1997).

As mentioned previously, the idea that organisms having simpler nervous systems, including invertebrates as well as vertebrates, are capable of some degree of conscious awareness has been advanced on behavioral grounds (Griffin, 1976; 1992; Rollin, 1998). Others have criticized the evidence for this proposition and made compelling arguments that there is nothing about the capabilities of most, if not all non-humans, that demonstrates the capacity for consciousness (Blumberg and Wasserman, 1995; Donald, 1991; Kennedy, 1992; Macphail, 1998). More pertinent to the present discussion is the critical issue of mechanistic feasibility. Conjectures that a different, nonneocortical mechanism for consciousness might exist, are contradicted by extensive neurobiological evidence. As explained earlier, the neural processes mediating conscious awareness appear to be highly complex, requiring large, structurally differentiated neocortical regions with great numbers of exactly interconnected neurons (Tononi and Edelman, 1998). What is more, the type of neocortex most essential to consciousness, the nonsensory association cortex, comprises the vast majority of human cerebral cortex, but it is a very small proportion of the neocortex in most mammals (Mountcastle, 1998; Deacon, 1992a). Consequently, conscious experience resembling that of humans would be extremely improbable for the great majority of mammals. Even great apes, having substantially less nonsensory association neocortex than humans (Deacon, 1992a), would be unlikely candidates for human-like higher-order consciousness, as their behavioral characteristics, such as inability to acquire true language use, indicate (Donald, 1991; Macphail, 1998).

As the foregoing presentation has explained, the brains of fishes are profoundly lacking in both the quantitative and qualitative structural features required for the generation of consciousness as we understand it. Furthermore, fish brains are understood well enough to make it highly implausible that there are alternate, functionally uncommitted systems that could meet the requirements for generation of consciousness, namely, exceptionally high interconnectivity within the cortex and between the cortex and thalamus, and enough nonsensory cortical mass and local functional diversification to permit regionally specialized, differentiated activity patterns (Edelman and Tononi, 1999). In addition, despite the great differences between fishes and humans at the level of the cerebral cortex, the organization of the brainstem and subcortical cerebrum has much fundamental similarity (Wullimann,1998). This similarity stems, of course, from a shared vertebrate genotype. Consequently, many shared neurobiological problems in mammals and other vertebrates tend to have conserved, common neurobiological solutions. Several examples illustrate this point. Locomotor patterns are generated by the spinal cord and brainstem in fishes and mammals alike (Grillner *et al.*, 1998; Rose *et al.*, 2000). Neuroendocrine control issues from the hypothalamus to the pituitary in all vertebrates (Butler and Hodos, 1996). Identical neurochemicals, such as monoamines, are produced by neurons in corresponding vertebrate brainstem locations, and these neurochemical systems appear to serve similar, shared functions across species (Butler and Hodos, 1996; Nieuwenhuys *et al.*, 1998a).

Conspicuous exceptions to such vertebrate neural continuities occur in cases where an organism, through a separate evolutionary path, has acquired something fundamentally new, such as

language in humans. Even here, the new neural mechanism was probably founded on an expansion of less specialized cortical regions already present in primate ancestors (Preuss, 2000). These specialized human cortical regions, through genetic change, took on previously impossible functions. Furthermore, as inferred above, nothing about the behavior of a fish requires a capacity for conscious awareness for its explanation (Kennedy, 1992; Macphail, 1998). In contrast to the predictable, species-typical behavior of fishes, the immense diversity of human solutions to problems of existence through novel, mentally contrived strategies, is unparalleled by any other form of life. The full range of this behavioral diversity and flexibility depends on the capacity for consciousness afforded by the human neocortex (Kolb and Whishaw, 1995; Donald, 1991).

Another computer analogy is instructive here. Those of us who used desktop computers throughout their development during recent decades have seen greatly increased capabilities of these devices emerge with absolute dependence on changes in hardware and software. It is inconceivable that one could run Windows™, read and write to compact disks, and search the Internet, all at high speed and in high-resolution color, with a 1982 desktop computer that had minimal memory, no hard disk drive, and a monochrome monitor. The massive additional neurological hardware and software of the human cerebral cortex is necessary for the conscious dimension of our existence, including pain experience. However, as in fishes, our brainstem-spinal systems are adequate for generation of overt reactions. To propose that fishes have conscious awareness of pain with vastly simpler cerebral hemispheres amounts to saying that the operations performed by the modern computer could also be done by the 1982 model without additional hardware and software.

Thus, fishes have nervous systems that mediate effective escape and avoidance responses to noxious stimuli, but, these responses must occur without a concurrent, human-like awareness of pain, suffering or distress, which depend on separately evolved neocortex. Even among mammals there is an enormous range of cerebral cortex complexity. It seems likely that the character of pain, when it exists, would differ between mammalian species, a point that has been made previously by pain investigators (Melzack and Dennis, 1980; Bermond, 1997). Bermond has critiqued claims that non-human species can experience pain and suffering and argued that because conscious awareness of pain depends on extensively developed frontal lobe neocortex, few (if any) mammals besides humans possess an adequate cortical substrate for pain experience.

X. THE REACTIONS OF FISHES TO NOCICEPTIVE STIMULI ARE SIMILAR TO THEIR REACTIONS TO PREDATORS AND OTHER NON-NOCICEPTIVE STIMULI

Humans are acquainted with behavioral responses of fishes through our attempts to capture them. For example, when a fish is hooked by an angler, it typically responds with rapid swimming behavior that appears to be a flight response. Human observers sometimes interpret this flight response to be a reaction to pain, as if the fish was capable of the same kind of pain experienced as a human. From the foregoing explanation, it should be clear that the fish's behavior results largely from brainstem and spinal patterns of activity that are elicited by being hooked. However, the flight responses of a hooked fish are essentially the same as responses of a fish that is being pursued by a visible predator or a fish that has been startled by a vibration in the water. These visual and vibratory stimuli would not activate nociceptive types of sensory neurons. Consequently, the flight responses of fishes are actually evoked by many types of stimuli, including those that do not activate nociceptive neural systems. Recall, that elasmobranchs make vigorous "flight" responses to being hooked in spite of being ill-equipped to process nociceptive stimuli. Thus, the escape responses of a fish cannot be taken to represent a specific response to nociception. Instead, they represent protective responses to a range of stimuli associated with predators or other

threats, to which a fish responds with innate behavior programs and possibly nonconscious, implicit learning.

It might be proposed that the fish's responses to being hooked or pursued by a predator are indications of a fear response. This proposal leads to the same type of analysis that was applied to the psychological experience of pain. There is a close parallel between current neuroscientific nderstanding of pain as well as emotions like fear: the experience of fear is also a conscious psychological phenomenon that, similar to pain, requires an adequate neocortical system to be felt. Emotional reactions, like nociceptive responses, are generated by subcortical brain systems. The principal brain region that generates fear reactions is the amygdala, a subcortical structure deep in the temporal lobe (Kolb and Whishaw, 1996; LeDoux, 1996; Price, 1998; Strauss *et al.*, 1982). However, there is no experienced dimension to such emotional responses unless they become registered at the neocortical level that supports consciousness (Dolan, 2000; LeDoux, 1996; Öhman *et al.*, 2000). This understanding of the neurology of emotion has been succinctly expressed by Joseph LeDoux (1996):

> Emotional feelings result when we become consciously aware that an emotion system of the brain is active. Emotions evolved not as conscious feelings... but as brain states and bodily responses. The brain states and bodily responses are the fundamental facts of an emotion, and the conscious feelings are the frills that have added icing to the emotional cake.

Fishes and other animals without the extensive nonsensory association neocortex that is required to support consciousness may be capable of certain basic behaviors and physiological reactions that fit the functional definition of emotional responses. However, these responses, like nociceptive responses, occur without a felt, experiential dimension. The brain systems known to be necessary for the experience of fear or other emotional experiences are not present in fishes.

XI. PREVIOUS STATEMENTS CONCERNING THE ABILITY OF FISHES TO EXPERIENCE PAIN

There have been diametrically opposed statements made concerning the capacity of fishes to experience pain, with writers disputing this capacity (LaChat,1996; Macphail, 1998) and others asserting it, for example, Bateson (1992) Gregory (1999), Stoskopf (1993, 1994); Verheijen and Flight (1993, 1997). The arguments presented in support of the belief that fishes are capable of feeling pain appear to take three forms: (1)that behavioral reactivity to presumed noxious stimuli is evidence of pain perception; (2) that learning by fishes to avoid noxious stimuli or to develop Pavlovian conditioned responses to these stimuli is evidence of pain experience; and (3) that the same or equivalent neural structures and neurochemicals mediating pain in humans are present in fishes. The following presentation will show that none of these arguments is valid.

The first argument, equating behavioral reactivity with pain experience, obviously fails to distinguish nociception or nociceptive responses from pain experience. This misinterpretation, fueled by anthropomorphic thinking, is rampant in the culture at large and much of the scientific community. However, as this article has explained at length, the separateness of nociception and nociceptive behaviors from the psychological experience of pain is a well-established principle in the scientific literature. What apparently is not understood by those who advance this argument is that the neural mechanisms generating nociceptive behaviors operate at lower levels of the nervous system and run their course regardless of whether there is a higher level where the conscious experience of pain is produced. Because the higher brain level responsible for awareness of the sensory and emotional dimensions of pain does not exist in fish brains, all of their neurobehavioral reactivity to noxious stimuli is nociception and not pain. In addition, the preceding section made the point that

the responses of fishes to nonnociceptive and nociceptive stimuli may be very much alike. This point further illustrates the fact that an anthropomorphic, face value interpretation of responses of fishes to noxious stimuli is invalid. The notion of sentience is sometimes used by proponents of the argument that fishes can experience pain. This word is used to mean that reactivity denotes conscious awareness of stimuli (Fox, 1986). Thus, the word's broad application to simple and complex organisms, alike, conveys an unsubstantiated attribution of a higher-order neuropsychological capacity. Of course, the notion of sentience also fails to distinguish nociception from pain. This archaic word is very misleading, with invalid connotations that are granted without critical appraisal. It has little place in scientific discourse.

The second argument assumes that the presence of learning signifies a capacity for conscious awareness of the noxious stimuli used to reinforce that learning. As has been explained, instrumental and Pavlovian conditioning are forms of associative, implicit learning that occur in fishes and diverse other vertebrates and invertebrates. As cases of implicit learning, they operate without awareness (Macphail, 1998) and constitute no evidence of awareness of pain or any other experience. Evidence of learning to avoid noxious stimuli has been presented as an argument that the behavior of the fish is more than a reflexive response (Verheijen and Flight, 1997). While this is true, many complex, nonreflexive behaviors are done without consciousness, even in humans (Macphail, 1998), so the presence of complex behaviors in response to nociceptive stimuli is not evidence of awareness of pain. The term "fear" conditioning has also been used to suggest that a conscious awareness of fear is involved in the learning process (Davis and Klinger, 1994). In fact, "fear" conditioning paradigms are always examples of implicit learning and no awareness of "fear" is necessary for their occurrence (Dolan, 2000).

The third argument takes various forms. In one form it is stated that fishes have spinal cord and brainstem pathways similar to those that transmit "pain" in humans or other mammals. It is also stated that fishes have many of the same neurotransmitters and neuromodulators, such as endogenous opioid neuropeptides, that are present in mammalian spinal and brainstem nociception systems (Gregory, 1999; Stoskopf, 1994). The conclusion drawn from this evidence is that fishes must be capable of experiencing pain. It is evident that the spinal cord and brainstem of fishes do have similar neuroanatomical components, such as ascending spinoreticular/ spinothalamic pathways, that are utilized for processing nociceptive information (Nieuwenhuys *et al.*, 1998a; Wulliman, 1998). To the extent that a fish or any other animal can react to nociceptive stimuli, there must be a neural system meditating such reactions. However, in the absence of an adequate neural substrate for generating consciousness and awareness of the pain experience such as an extensive frontoparietal neocortical system, the behaviors evoked by nociceptive stimuli are performed without conscious awareness. The effectiveness of analgesics such as morphine for reducing behavioral responses of fish to noxious stimuli has also been given as an argument for a capacity for pain experience (Gregory, 1999; Stoskopf, 1994). Of course, as has been discussed already, opiates act on lower levels of the nervous system to reduce nociceptive responsiveness, so opiate effects constitute no evidence of a capacity for pain experience. The presence of endogenous opioids in fish and the behavioral effectiveness of opiate analgesics does suggest that these endogenous opioids might attenuate nociception in fishes as they do in mammals. Having said this, one must again point to the case of elasmobranchs, where the capacity for nociception is doubtful. In these fishes some of the typical components of the nociceptive system are present, such as the ascending lateral spinal pathway and the peptides substance P and encephalin in the spinal gray matter (Snow *et al.,* 1993). These neuropeptides are thought to be a critical facilitator and attenuator, respectively, of nociceptive neural transmission. Neuropeptides tend to serve multiple functions, however, such as promoting vasodilation and healing (Strand, 1999), and it would appear that these peptides must be serving nonnociceptive functions in elasmobranchs (Ritchie and Leonard, 1983).

Another variation on the argument of neurostructural similarity is the claim that forebrain structures believed to be important for pain experience in humans have homologies in fishes (Verheijen and

Flight, 1997). This assertion misrepresents what is known about fish forebrain structure. The relationship between cerebral hemisphere structures of fishes and those of mammals has been debated throughout the history of comparative neuroanatomy (Nieuwenhuys *et al.*, 1998a). Compared with mammals, the cerebral hemispheres of fishes are very poorly differentiated, making it extremely difficult to define structural subdivisions with confidence, much less show that these subdivisions correspond to specific regions of a mammal's brain. Two classes of structures are at issue here: limbic structures and neocortex. Limbic structures are a collection of subcortical nuclear and cerebral cortical structures that are believed to have a role in generation of emotion and a variety of life-sustaining behaviors, including reproduction, aggression and defense, feeding, and drinking. Because of the limited structural differentiation of fish cerebral hemispheres, determining if specific limbic system components such as the amygdala are present, has long been unresolved. It now seems agreed among neuroscientists that some mammalian limbic structures such as the septal region and the amygdala have homologous structures in fish brains. The presence of structural homologies, however, cannot be taken as evidence that the emotional reactivity and pain experience of humans and fishes is similar. First, it must be emphasized that homology only means that a structure is believed to have been present in a common ancestor of fishes and mammals (Butler and Hodos, 1996). No functional equivalency is established by neuroanatomical homology. In fact, functional equivalency of such structures is impossible because the amygdala in fishes lacks the diverse structural subdivisions and specific connections, such as frontal lobe neocortex, that are critical to the functions the human amygdala. In addition, increasing evidence shows that activity in the amygdala is not consciously perceived unless this activity also registers in the neocortex (e.g., Dolan, 2000). So, even if a functionally equivalent amygdale were present in a fish brain (which it is not), the absence of a neocortical mechanism for consciousness would prevent it from generating "fear" or any other consciously experienced emotion.

In contrast to previous assertions (Verheijen and Flight, 1997), a specific role for of the amygdala in pain is not established (Price, 1999). More pertinent to this discussion is the cingulate gyrus. As previously discussed, this structure appears to be critical for the emotional aversiveness of pain, but differing from most other limbic structures it is identifiable only in mammals, probably because its structure depended on the evolution of neocortex.

The structural homology argument has also been extended to neocortex. It has been denied that "cortex" is a recent evolutionary acquisition that ought to distinguish fishes from humans (Verheijen and Flight, 1997). This argument is another straw man, because neocortex is the only type of cortex in question and its absence in fishes is an undisputed neuroanatomical fact (Allman, 1998; Butler and Hodos; 1996; Northcutt and Kaas, 1995; Nieuwenhuys *et al.*, 1998b). It has also been implied that dependence of a particular type of avoidance learning in a fish on the telencephalon (e.g., cerebral hemispheres) proves that fishes are aware of nociceptive stimuli (Gregory, 1999). This interpretation is invalid, of course, because avoidance conditioning occurs unconsciously and is not evidence of awareness of pain or any other experience.

There is also a more general argument assuming that pain is poorly understood, and facts critical to answering the question of pain experience in fishes are either unknown or unknowable. Given this view, it is assumed that the best course is to err on the side of believing that fishes can experience pain (Stoskopf, 1994, 1993). It should be evident clearly from the foregoing presentation that pain and its neurological basis have been under intense and productive investigation for decades and the resultant body of empirical data have clear implications for the question of pain in fishes. The issue of greatest concern is whether fishes can experience pain and suffering in a way that resembles our experience, that is, do fishes have a capacity to suffer that meaningfully approximates the psychological impact of pain-induced suffering in humans? A large and diverse array of empirical evidence addressing this issue has been presented. This evidence identifies, with a high degree of certainty, the neurological requirements that are essential for the conscious experiences of pain and suffering. These requirements consist of an extensive system of functionally differentiated neocortical structures that underlie both consciousness and the

psychological dimensions of pain experience. There is no similar system in fish brains, nothing with the structural or functional capability of neocortex, much less human neocortex. This fact must be appreciated in light of one of the most well-substantiated principles of neuroscience: that functions of nervous systems, including psychological functions, depend on specific structural features of these nervous systems.

A related argument is that a fish can not be asked if it feels pain; therefore, it is unknowable if it does or not. The fact that an indirect approach is needed to answer the question of pain experience by fishes is neither ground for suspending judgment or an unusual circumstance in science. The clinical identification of brain death provides an instructive illustration. The structural and functional conditions required for a living, viable brain are known. If diverse tests reveal that these conditions do not exist, the diagnosis of brain death, with its momentous implications, is made (Young *et al.*, 1998). Although it's impossible to talk to the victim to make a determination of brain death, there is a high degree of certainty about the diagnosis.

XII. FISHES RESPOND TO NOXIOUS STIMULI WITH STRESS RESPONSES

Regardless of the probable absence of the psychological experience of pain or fear in fishes, these organisms are still neurologically well designed to react to injurious or threatening stimuli with defensive responses. The vertebrate nervous system has exquisite features for mediating predator-avoidance and escape from threatening stimuli, as well as compensatory physiological and neuroendocrine stress responses. These defensive responses have well-documented health and well-being implications for fishes (Iwama *et al.*, 1997). It is at this level of function that concerns about reactivity to nociceptive and other stress-inducing stimuli should be addressed.

As mentioned above, one salient vertebrate neurological common denominator is the neuroendocrine system, which includes the subdivision that mediates the stress response (Sumpter, 1997). In humans and fishes alike, neural activity evoked by noxious stimuli enter the brain from diverse sensory pathways and ultimately activate hypothalamic neurons that initiate neuroendocrine and physiological stress responses. In fishes, stressful stimuli evoke a suite of immediate responses, including hormonal events, such as cortisol and catecholamine release, as well as various behavioral responses (Schreck *et al.*, 1997). In humans, as explained above, there is no awareness of purely subcortical brain events. Thus, the subcortical processes that control our endocrine and physiological stress responses occur unconsciously. Also, we may or may not be consciously aware of the provocative environmental events that constitute the stressors, because some emotionally provocative types of stimuli are processed subcortically without our awareness (Dolan, 2000; LeDoux, 1996; Öhman *et al.*, 2000). In many cases, of course, we are aware of pain-provoking or emotionally distressing events that cause endocrine stress responses because these events generate adequate cortical activity for conscious awareness. However, the foregoing argument supports the conclusion that stress responses in fishes, such as behavioral reactions to noxious stimuli, are produced without conscious awareness of the eliciting stimuli, even though such stimuli might trigger behavioral avoidance or physiological stress responses. Thus, fishes would undergo stress without emotional distress.

Nonetheless, endocrine stress responses, especially sustained or repeated ones, can undermine the health and well being of fishes (Iwama *et al.*, 1997). In addition, when fishes are used in research, their physical well-being is of great importance for the collection of sound scientific information. Considerations of the welfare of fishes in research, or other contexts such as aquaculture, should focus on reducing exposure of fishes to conditions that provoke stress or undermine their physical well-being rather than on concerns about human-like psychological emotional distress, of which fishes are neurologically incapable.

SUMMARY AND CONCLUSIONS

There are widely disparate beliefs concerning whether fishes can experience pain and suffering. These beliefs influence policies governing the diverse uses of fishes. Given these differences in understanding and opinion, the extensive contemporary knowledge on the neural basis of pain and the neurobehavioral nature of fishes has been reviewed to provide the first detailed analysis of whether fishes can experience pain and suffering. The human predilection for anthropomorphic interpretations of the behavior of non-humans tends to undermine this type of inquiry. Human centered perspectives on fishes are inappropriate because the evolutionary histories of fishes and mammals have been separate for about 400 million years, and the appearance of the brain structures that are the basis for human-like experience is quite recent. A consequence of this long evolutionary separation is that the brainstem is the most conserved and similar brain feature in fishes and humans, whereas the massive expansion of the cerebral hemispheres and development of neocortex in humans, and other mammals, sets these species substantially apart from fishes.

The neurological basis of human consciousness is becoming increasingly well understood and is known to depend on functions of the neocortex. All forms of consciousness in humans depend specifically on the presence of expansive regions of "association" cortex, regions that are the most developed in humans. Neural activity confined to subcortical brain regions such as the brainstem is inaccessible to consciousness.

The behavior of fish species is highly diverse and often complex, but it is also stereotyped and species typical. Nonetheless, fishes, similar to all vertebrates and diverse invertebrates, are capable of associative, implicit learning, a type of learning that occurs without conscious awareness. The great majority of nonolfactory behaviors shown by fishes, including responses to noxious stimuli, can be performed after removal of the cerebral hemispheres. Thus, the evolution of fishes has resulted in a mode of adaptation that is served by neurobehavioral processes controlled largely by the brainstem and spinal cord. This stands in contrast with the great dependency of mammalian behavior, especially that of humans, on the cerebral hemispheres.

Whether the neocortex of non-human mammals can support a rudimentary type of consciousness is not entirely clear. While some argue that only humans are capable of consciousness, others propose that some rudimentary form of consciousness exists in nonmammalian vertebrates, even invertebrates. The latter claim has been made without the rigor of a mechanistic analysis. The need for such a mechanistic analysis is a critical issue because increasing evidence shows consciousness to depend on extensive regions of higher-order, functionally unique, nonsensory neocortex. The neocortex of most mammals is predominantly sensory cortex and non-mammals have no neocortex or any equivalent neural system.

In humans, pain is a psychological experience with a sensory-informational dimension and a dimension of emotional unpleasantness or suffering. These components of pain are experienced only in a conscious individual and depend on processes involving the somatic sensory regions of the neocortex (sensory dimension) and extensive nonsensory cortical regions of the frontal and parietal lobes (emotional dimension). In distinction to the neocortically based conscious experience of pain, all of the neural processing of noxious stimuli by peripheral nerves, the spinal cord, the brainstem, and other subcortical regions of the brain, occurs without conscious awareness. This subcortical processing is designated "nociception" to distinguish it from the conscious awareness of pain. In all vertebrates, including humans, innate responses to nociceptive stimuli, such as limb withdrawal, facial displays, and vocalizations are generated by neural systems in subcortical levels of the nervous system, mainly the spinal cord and brainstem. Understanding that the display of behavioral responses to nociceptive stimuli does not, by itself, imply conscious awareness of pain is

vital for a valid conceptualization of the neural basis of pain. For example, humans that are completely unconscious due to massive damage of the cerebral cortex can still show facial, vocal, and limb responses to nociceptive stimuli even though the experience of pain is impossible. Fishes with the cerebral hemispheres removed exhibit essentially normal responses to noxious stimuli.

Nociceptive reactions to noxious stimuli are a universal characteristic of animal life, even in simple invertebrates that have no brains, a fact demonstrating that reactivity to noxious stimuli does not imply conscious awareness. The nociceptive processes of fishes have received much less study than those of mammals, but it is clear that there are major differences as well as some similarities between fishes and mammals in neural systems that might mediate nociception. Sharks and rays show a conspicuous absence of the essential peripheral nerve and spinal cellular components of nociception, which may explain their relative behavioral unresponsiveness to injury. Teleosts have peripheral nerve, spinal, and brainstem structures more similar to those mediating nociception in mammals. However, the brain level of most importance for the conscious awareness of pain in humans, an extensive frontal and parietal lobe neocortex, is completely absent in fishes. The cerebral hemispheres of fishes have only a more simple type of cortex that lacks the structural complexity, massive interconnectivity, and spatial extent of neocortex, the cortex necessary for pain experience. There is no alternate neural system that could provide another, functionally comparable, mechanism for pain experience in fishes. The same conclusion applies to emotions such as fear. The neural structures known to be important for conscious emotional experiences, which include extensive neocortical regions such as those involved in pain, are not present in fishes, aside from very rudimentary homologues, which could not mediate conscious emotional experiences.

It is a well-established principle in neuroscience that neural functions depend on specific neural structures. Furthermore, the form of those structures, to a great extent, dictates the properties of the functions they subserve. If the specific structures mediating human pain experience, or very similar structures, are not present in an organism's brain, a reasonably close approximation of the pain experience can not be present. If some form of pain awareness were possible in the brain of a fish, which diverse evidence shows is highly improbable, its properties would necessarily be so different as to not be comparable to human-like experiences of pain and suffering.

Claims for and against the possibility that fishes can experience pain have been published, but none have considered the full range of evidence presented in the present review. Arguments that fishes are capable of experiencing pain and suffering have mainly taken three forms. First, behavioral reactivity to injurious or presumed noxious stimuli has been taken as prima facie evidence of conscious pain experience. Obviously, this interpretation confuses nociceptive behavioral responses, which are mediated by subcortical, and, hence, nonconscious levels of the nervous system, with subjective, conscious experiences that depend on neocortical brain regions that fishes do not have. Second, it has been asserted that learning by a fish to avoid noxious stimuli or to develop Pavlovian conditioned responses to these stimuli is evidence of pain experience. This interpretation fails to acknowledge that these types of implicit learning occur unconsciously and, thus, constitute no evidence of awareness of pain. Third, it has been claimed that the same or equivalent neural structures as those known to mediate pain in humans are present in fishes. This assertion is clearly invalid for elasmobranchs and is only partly correct for teleosts. The latter species have peripheral nerve, spinal, and brainstem components for nociception similar to those present in mammals. However, the essential neural components for pain experience, extensive frontal and parietal neocortical regions, are not present in fishes. It has been claimed by some that the fish cerebrum has a cortex equivalent to neocortex, but this claim is strongly contradicted by extensive empirical evidence. In addition, the presence of "pain-related" endogenous opioid neuropeptides, or of analgesic actions of opiate drugs in fishes, is not evidence of pain experience because the actions of these compounds are principally at lower, subcortical levels of nociceptive processing.

The fundamental neural requirements for pain and suffering are now known. Fishes lack the most important of these required neural structures, and they have no alternative neural systems for producing the pain experience. Therefore, the reactions of fishes to noxious stimuli are nociceptive and without conscious awareness of pain. The evidence supporting this conclusion is extensive and diverse, thus permitting a high degree of confidence in its correctness. In view of the weight of the evidence presented here, any future proposal for the existence of pain awareness in fishes, or neurologically comparable vertebrates, should provide a compelling empirical basis to justify its consideration. Such a proposal must address the mechanistic plausibility of any hypothesized alternate neural basis for pain experience. The proposal must also show that some aspect of the response of fishes to nociceptive stimuli requires pain awareness, rather than just nociception, for its explanation.

Although it is concluded from the foregoing analysis that the experiences of pain and emotional distress are not within the capacity of fishes, this conclusion in no way devalues fishes or diminishes our responsibility for respectful and responsible stewardship of them. Fishes constitute a highly evolved, diverse, and complex life form whose history on the Earth vastly eclipses the brief existence of humans. Our diverse uses of fishes have ancient historical precedents and modern justifications, but our increasingly deleterious impacts on fishes at the population and ecological levels require us to use our best scientific knowledge and understanding to foster their health and viability.

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2.2.

A critique of the paper: "Do fish have nociceptors: Evidence for the evolution of a vertebrate sensory system" published in Proceedings of the Royal Society: Biological Sciences. 270(1520):1115-1121, 2003 by Sneddon, Braithwaite and Gentle (http://fidelio.ingentaselect.com/vl=7787424/cl=12/nw=1/rpsv/cgi-bin/linker?reqidx=/cw/rsl/09628452/v270n1520/s2/p1115.idx&lkey=-182115764&rkey=563874900) .
http://www.catchword.com/cgi-bin/linker?ini=rsl&reqidx=/cw/rsl/09628452/v270n1520/s2/p1115

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The paper by Sneddon, et al. is flawed and does not provide any legitimate evidence that trout are capable of feeling pain. There are numerous problems with methods and data interpretation in this paper but this critique will focus only on those of greatest significance. First, an explanation of the invalid claims for evidence of pain will be presented, followed by an account of the misinterpretations of the behavioral results.

Flaws in the argument for a demonstration of pain.

1. **The authors' definitions of pain and nociception are invalid, consequently this paper does not actually deal with pain (a conscious experience), it deals only with nociception (unconscious responses to noxious stimuli**). Pain, as defined by the International Association for the Study of Pain is purely a conscious experience, with a sensory component and a component of emotional feeling (suffering). In contrast to this conscious experience of pain, the unconscious detection, transmission and response to noxious stimulation by lower levels of the nervous system is and defined as **nociception - not pain**. According to Sneddon, and associates, any behavior that is a reflex would be evidence of nociception but any behavior more complex than a reflex would be evidence of pain. This way of distinguishing pain from nociception is invalid because there are clearly complex, non-reflexive behaviors that can be purely nociceptive and unconscious. For example, humans with extensive damage of the cerebral hemispheres can still make a complex of responses including facial grimaces, vocalizations, struggling and avoidance reactions to noxious stimuli, but they are unconscious and unable to experience pain. From the definition of pain used by Sneddon and associates, it would be concluded that these unconscious humans are feeling pain rather than making purely unconscious, nociceptive responses, which is clearly erroneous. There are many other examples of complex, non-reflexive, even distress-like behaviors that can be performed unconsciously. A person having a night terror, for instance, will show a compelling fear-like display, including a scream, terrified facial expression, elevated heart rate, sweating and dilated pupils, even though they are unconscious and in such deep sleep that they are difficult to awaken. The point is that complex behavioral displays that seem to reflect distress can be purely unconscious – even in humans. It should not be hard to appreciate that the behaviors of which a fish is capable could be unconscious as well.

2. **In order to show that a fish experiences pain, it is necessary to show that a fish has consciousness. Without consciousness, there is no pain.** None of the fish behaviors in this paper require the involvement of consciousness and the authors don't even deal with this essential issue. Furthermore, as I have shown in my 2002 Reviews in Fisheries Science paper, there is extensive scientific evidence that pain

and consciousness depend on very specific brain regions, namely specialized neocortex regions of the cerebral hemispheres. These specialized neocortical regions perform the additional levels of neural processing, beyond unconscious nociception, that make the experience of pain possible. These brain regions are absent in fishes and there are no alternative brain systems to perform the same functions. Consequently, there is no neurological basis for assuming that a fish might have a capacity for consciousness or pain. Thus, the burden of proof that trout are conscious and potentially capable of feeling pain remains on these authors. They dealt with this issue only by citing previous studies that also used invalid criteria for pain, such avoidance learning, which actually occurs unconsciously. Only anthropomorphic speculation would lead one to conclude that the trout in this study were experiencing pain.

The behavioral results allegedly showing evidence of pain were misinterpreted.

1. The behavioral studies were done by injecting large volumes of one of three solutions: bee venom, acetic acid solution or saline, into the jaw of rather small trout. For the sizes of the fish used, **these injections of liquid would have been equivalent to injecting 100 milliliters (more that 3 ounces) of solution into the lip of a human**. Bee venom contains a great variety of toxins that affect the nervous system and cause a hormonal stress response in addition to stimulating receptors signaling tissue injury. **In spite of the large dose of venom or acid, the activity level of these fish was not affected, they did not hide under a shelter in the tank and they resumed feeding in less than three hours.** Furthermore, fish that received no injection at all or fish that received a saline injection did not feed, on average, for an hour and 20 minutes, showing that a large saline injection produced no more effect than just handling. The acid and venom-injected fish also showed an infrequent rocking behavior that may have reflected a difficulty by the fish in maintaining an upright posture, given the magnitude of the toxic chemical trauma created by the injection. But, even if the infrequent rocking was a response to nociceptive stimulation of the mouth, there is no reason to believe that it is any more than an unconscious nociceptive response, rather than an indication of "pain".

2. Sneddon and associates also state that the acid-injected fish rubbed their mouths against the gravel (they don't say how often), but the venom-injected fish did not. They concluded that mouth rubbing was an indication of pain because mammals, including humans, rub injured tissues to alleviate nociceptive input. If so, why did the venom-injected fish, that were also supposed to be in pain, not perform this behavior? In addition, injections of irritants into skin tissues is known to cause hyperalgesia, where skin becomes hypersensitive, like the effect of a sunburn. Who rubs sunburned skin against gravel to alleviate the pain? At one point in the paper, Sneddon and associates say that feeding was suppressed because the fish were avoiding mouth stimulation, which would cause "pain." But later, they say that mouth rubbing was a way of reducing "pain." These are contradictory interpretations and you can't have it both ways. Their interpretations of the mouth-rubbing behaviors don't make sense nor do they show conscious experience of pain.

3. One of the few effects actually produced by the acid or venom injections was an elevated opercular beat rate (breathing). This response could have resulted directly from gill irritation due to leakage or blood borne spread of the acid or venom injections, but even if increased opercular beat rate was due to nociceptive stimulation of the mouth, this unconscious movement proves nothing about conscious pain.

4. One caveat regarding the behavioral data described above is the fact that some of the statistical analyses were not done correctly. Data for opercular beat rate and for time to resume feeding were analyzed by one-way analysis of variance, but conclusions were made about specific group differences in these measures. With this type analysis, it is not legitimate to conclude that one group (e.g. acid or venom injected differed from any other group (e.g. handeled or saline injected), but the authors made such

conclusions, nonetheless. Given the sizes of the standard errors of the means for these data, however, the group differences reported by the authors would probably have been substantiated following proper statistical analysis.

To summarize, the most impressive thing about the acid and venom injections was the relative absence of behavioral effects, given the magnitude of the toxic injections. How many humans would show little change in behavior or be ready to eat less than three hours after getting a lemon-sized bolus of bee venom or acid solution in their lip? Rather than proving a capacity for pain, these results show a remarkable resistance to oral trauma by the trout. It comes as no surprise, then, that many anglers have had the experience of catching the same fish repeatedly within a span of a few minutes. Of course predatory fishes, including trout, feed avidly on potentially injurious prey like crayfish, crabs and fish that have sharp spines in their fins – which further indicates that these fish are not highly reactive to noxious oral stimuli.

In addition, Sneddon and associates claim to have presented the first evidence for nociceptive sensory receptors in fish, but their results were neither wholly original nor unexpected. In my 2002 Reviews paper, I cited a 1971 study by Whitear that showed the presence of C-fibers in fish. C-fibers are a principal type of nociceptive receptor, so there was very good reason to assume that trout would have nociceptive receptors. Another technical issue arises in the authors' description of their procedure for decerebration of trout in order to make them "insentient." The term sentience is vague and has no standard scientific meaning, but apparently Sneddon, et al. were performing this decerebration in order to eliminate any potential pain that they assumed was within the capacity of the trout. The usual means of producing a decerebration is to remove all brain tissue above the midbrain. According to Sneddon, et. al, however, they removed the "...olfactory and optic lobes and cerebellum..." This is peculiar and counterproductive because the entire pathway for nociceptive information from the periphery through the brainstem to the cerebral hemispheres would have remained intact in these fish, since the "ofactory lobes" but not entire cerebral hemispheres would have been removed according to this description. If fish could feel pain, as the authors contend (and I dispute), these fish probably would have.

The bottom line of this critique is that any attempt to show pain in fish must use valid criteria, including proof of conscious awareness, particularly a kind of awareness that is meaningfully like ours. This is not something that can be taken for granted, because on neurological and behavioral grounds it is so improbable that fish could be conscious and feel pain. Furthermore, the behavioral results of this study show that in spite of very large injections of acid solution or venom, the fish showed little adverse effect, hardly supporting the claim that they were in pain.

I wish to emphasize that the improbability that fish can experience pain in no way diminishes our responsibility for concern about their welfare. Fish are capable of robust, unconscious, behavioral, physiological and hormonal responses to stressors, which if sufficiently intense or sustained, can be detrimental to their health.

Cited reference: Rose, J. D. 2002. The neurobehavioral nature of fishes and the question of awareness and pain. Reviews in Fisheries Science, 10: 1-38. This paper can be obtained in electronic form from the author.

For another neuroscientist's critique of the Sneddon, et al., article, see:
http://www.spiked-online.com/articles/00000006DD91.htm

FISHERIES SOCIETY OF THE BRITISH ISLES
BRIEFING PAPER 2
FISH WELFARE

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EXECUTIVE SUMMARY

Preamble: Those who work professionally with fishes, whose sports or hobbies involve fish and those concerned with the welfare of animals in general seek answers to a number of questions about the effects of human activities on fish welfare. This Briefing Paper considers how welfare is defined and measured, and examines how various human activities affect fish welfare. No opinion is given about what is acceptable and what is unacceptable, but based on current knowledge harmful effects are identified so that others can make their own informed judgements.

Section 1: "Animal welfare" is a difficult term to define precisely. Different definitions focus on an animal's condition, on its subjective experience of that condition and/or on whether it can lead a natural life. These various definitions are not right or wrong, but simply highlight different aspects of welfare. The contents of this paper reflect all of these facets of welfare in fish.

Section 2: A big unresolved and controversial issue in welfare research is whether non-human animals experience what humans would call suffering when they are exposed to adverse events such as physical injury or confinement. A part of the human brain (the neocortex) generates the subjective experience of suffering. Fish brains lack this structure, so fish clearly cannot suffer in exactly the same way as we do. However, other parts of the fish brain are well developed and are used to produce complex behaviour, so lack of a neocortex does not mean that fish cannot experience some kind of suffering. Recent studies suggest that fish have the capacity to perceive painful stimuli and that these are strongly aversive. Consequently, injury or experience of other harmful conditions is a cause for concern in terms of welfare of individual fish.

Section 3: Some of the human activities that could potentially have negative effects on fish welfare are listed, including anthropogenic changes to the environment, commercial fisheries, recreational angling, aquaculture, keeping ornamental fish and scientific research. If an activity does cause harm to fish welfare, this does not necessarily mean that it should be stopped; the harm represents a cost that should be minimised and weighed against the benefits of the activity concerned.

Section 4: Wild fish experience a variety of adverse conditions, from attack by a predator or another fish of the same species to failure to find food or exposure to poor environmental conditions. This does not make it acceptable for humans to impose the same conditions on fish, but it does suggest that fish will have mechanisms to cope with these environmental challenges.

Section 5: Fish respond to such challenges via a physiological stress response - secretion of the "stress hormones" adrenaline and cortisol into the bloodstream. These induce short term (secondary) metabolic changes that make the fish better able to cope with the challenge. Longer-term (tertiary) effects of chronic stress include suppressed behaviour, immune function, growth and reproduction. Behavioural responses are an important part of the stress response, since they enable animals to avoid or overcome the stressor.

Section 6: There is no simple link between physiological stress responses and welfare. It is perhaps unlikely that short-term adaptive responses to challenge cause suffering, but tertiary responses to prolonged, chronic stress are indicative of poor welfare. A number of indicators (based on physiological stress, general health and behaviour) can be used to assess fish welfare, both in a scientific and in a practical context. None is perfect and the best strategy is to use as many as possible and look for robust effects.

Section 7: An overview of the available information tells us that various human activities can harm fish welfare. It also tells us that such effects depend on the species concerned (for example, farmed Arctic charr do better at high densities but farmed trout do worse) and that they are context-dependent (for example, negative effects of stocking density may disappear if water quality is good).

Section 8: It can be misleading to extrapolate from what we know about the welfare of mammals and birds to fish, because fish are a different in ways that are important when considering their welfare. They do not need to fuel a high body temperature, so effects of food deprivation on welfare are not so marked. For species that live naturally in large shoals, low rather than high densities may be harmful. On the other hand, fish are in intimate contact with their environment through the huge surface area of their gills, so they are particularly vulnerable to poor water quality and pollution. General criteria for welfare of other vertebrate animals need to be modified to accommodate these facts before they can be usefully applied to fish.

Section 9: The scientific study of fish welfare is at an early stage compared to work on other vertebrates and a great deal of what we need to know is yet to be discovered. More information is needed about general issues, such as what it means to say that a fish is suffering, and about specific issues, such as the effects of particular activities in particular species. Some areas where more research is needed are:

- The behavioural responses of fish to harmful stimuli and the neural mechanisms that cause these responses.
- The mental capabilities of fish and how measurable events such as physical damage generate subjective states of well-being or suffering.
- Possible 'behavioural needs' that fish must be able to express.
- Diseases in fish and the relationship between health and welfare.
- The development of low-tech, easily applied indicators of fish welfare.
- The welfare of ornamental fish and those held in display aquaria.
- The effects of human activities on welfare in species other than salmon and trout.
- The exact mechanisms by which the adverse effects of human activity come about.

Conclusions: Fish are sophisticated animals, far removed from unfeeling creatures with a 15 second memory of popular misconception. They are also different from birds and mammals in important ways. A heightened appreciation of both these points in those who exploit fish and in those who seek to protect them would go a long way towards redressing some current shortcomings in fish welfare.

1. INTRODUCTION

Aims and intended audience

The aim of this Briefing Paper (which has been prepared by a team with expertise in veterinary medicine, endocrinology, neurobiology, aquaculture, animal behaviour and fish biology) is to give an account of current understanding on a number of issues relating to fish welfare - what it means, why it matters, what humans do that may compromise it and how, in practical terms, it might be measured. We concentrate on the impact of human activity on welfare at the level of the individual, as opposed to the population, the species or the ecosystem and address the experiences of living animals and not the rights and wrongs of killing animals. Our intention is not to make judgements about what is right and what is wrong; instead it is to provide readers with the information needed to make informed decisions on this point. The document is aimed at a broad, non-specialist audience, including scientists in disciplines where fish welfare is relevant but may not be given appropriate consideration, legislative and regulatory bodies who may be more experienced in the welfare of mammals and birds than fishes, welfare and conservation groups, organisations or industries that use live fishes and members of the general public.

1.2 Why is animal welfare a cause for concern?

Animal welfare is the subject of intense debate and attitudes to it are changing fast. In particular, the welfare of fish is coming increasingly to the forefront of public concern, witnessed by a number of web sites (e.g. www.vetmed.ucdavis.colu/CCAB/fish, www.vet.ed.ac.uk/animalwelfare/Fish/Contents), reports and commentaries on this topic (Kestin 1994 for the RSPCA, Lymbury 2002 for Compassion in World Farming, RSPA Welfare Standards for Farmed Salmon 2002) and by discussions at the European Council (Baeverfjord 1998). Increasing concern for welfare is also reflected in the activities of those using or studying fish. For example, the Angling Governing Bodies Liaison Group and the British Field Sports Council commissioned a review of the scientific literature on fish welfare (Pottinger 1995), the European Aquaculture Society has organised sessions on welfare in aquaculture at several recent conferences (Joyce 1996), a number of industrial aquaculture companies have altered their procedures and facilities to take account of fish welfare (for example by developing more humane methods of slaughter, Robb *et al.* 2000), fish producers associations have developed accreditation schemes that include fish welfare as a criterion (www.britishtrout.co.uk; www.scottishsalmon.co.uk), the ornamental fish industry has instituted a code of conduct that addresses, among other things, the welfare of aquarium fish (Davenport 1993, www.aquariumcouncil.org) and the Fisheries Society of the British Isles requires work published in the *Journal of Fish Biology* to adhere to a set of guidelines for the use of animals in research.

The question of why people should care about the welfare of animals raises complex moral issues that have been discussed from many perspectives (e.g. Rollin, 1993; Sandoe *et al.*1997; Fraser 1999; Heeger & Brom 2001). It is beyond the scope of this Briefing Paper to review all these issues; instead we take a simple working position that animal welfare matters, both for moral and practical reasons. In our view, as agents potentially causing animal suffering, people have both a moral obligation and, in many countries, a legal requirement, to respond to concern on this issue. In practical terms also, it is often in our selfish interest to consider the issue of animal welfare; for example, the welfare of wild fish may be a sensitive index of the quality of important water sources, poor welfare of animals used in scientific research means poor science and poor welfare of farmed fish often equates to poor production.

1.3 What does 'animal welfare' mean?

To discuss animal welfare objectively, we need a definition and this is not easy to produce because the concept is complex and the word is used in different ways (Dawkins 1998, Appleby 1999). Most definitions fall into one of three broad categories (Duncan & Fraser 1997, Fraser *et al.* 1997) and it is important to note that none of these is right or wrong; they simply capture different aspects of what the word means. The examples given in this paper reflect all of these facets of welfare.

Feelings-based definitions are set in terms of subjective mental states. Here, the requirement for good welfare is that the animal should feel well, being free from negative experiences such as pain or fear and having access to positive experiences, such as companionship in the case of social species. This use of the term

welfare obviously depends on the animal concerned having subjective experiences and our understanding what these are; this controversial point (Dawkins 1998) is discussed in Section 2.

Function-based definitions centre on an animal's ability to adapt to its present environment. Here good welfare requires that the animal be in good health with its biological systems functioning appropriately. This definition is based on things that are relatively easy to see and measure, but it rests on the assumption that an animal whose body is functioning well is in a good mental state, and the converse. Again, this is a complex issue that is discussed further below (in Section 8).

Finally, *nature-based definitions* arise from the view that each species of animal has an inherent biological nature that it must express. Here good welfare requires that the animal is able to lead a natural life and express its natural behaviour. This approach is based on something we can potentially measure (what animals do in the wild and in captivity), but it relies on the assumption that animals suffer if they cannot express the full repertoire of behaviour that they show in the wild, which is not necessarily the case. Much behaviour of wild animals is shown in response to adverse conditions (as when fleeing from a predator) and it is hard to argue that welfare is compromised if these responses are not evoked. In other cases, animals may be highly motivated to perform an action independent of its consequences and may suffer if deprived of the opportunity to do so. Chickens are strongly motivated to build nests (as opposed to having access to a completed nest) and will work hard for the opportunity to do so (Hughes *et al.* 1989); arguably then, nest-building reflects a behavioural need that must be met if the hen is not to suffer. To relate this to fishes, wild Atlantic salmon migrate long distances at sea. If this happens because fish leave an area when the local food supply is poor and stop swimming when they find a good place to feed, there is no reason to believe that the welfare of salmon is compromised when they are prevented from migrating, provided they have plenty of food. On the other hand, if they have an instinctive drive to move to new areas regardless of food supply, confinement might well cause suffering, even though fish in cages are able to swim continuously. At present we do not know enough to distinguish between these alternatives.

1.4 Criteria for welfare

Given the difficulty of finding a comprehensive, generally acceptable definition of animal welfare, some authors have concentrated on identifying conditions that must be fulfilled if an animal's welfare is to be considered acceptable. One influential framework (based on the 'five freedoms' defined in the UK Farm Animal Welfare Council) recognises five domains in which welfare may be compromised (Mellor & Stafford 2001). These domains were developed for the commoner farm animals, essentially birds and mammals, but fish were considered later (FAWC 1996). The relevance of these domains to fish welfare is discussed further in Section 8.

Domain 1. Water and food deprivation, malnutrition.

Animals should have ready access to fresh water and an appropriate diet in sufficient quantities and with a composition that maintains full health and vigour.

Domain 2. Environmental challenge

Animals should have a suitable environment, including shelter and a comfortable resting area, whether outdoors or indoors.

Domain 3. Disease, injury and functional impairment

Disease should be prevented or rapidly diagnosed and treated.

Domain 4. Behavioural/interactive restriction.

Animals should have sufficient space, proper facilities and where appropriate, the company of the animal's own kind.

Domain 5. Mental and physical suffering

Conditions that produce unacceptable levels of anxiety, fear, distress, boredom, sickness, pain, thirst, hunger and so on should be minimised.

Evaluation of the fifth domain for welfare is difficult, because it assumes that an animal's condition with respect to domains 1-4 is translated into subjective mental states that humans would describe as well-being or suffering. This raises a big unresolved issue in welfare research - whether in non-human animals events that compromise welfare as defined in domains 1-4 (such as severe food deprivation, confinement and physical injury) generate the mental state of suffering (conscious experience of something as very unpleasant, Dawkins 1998). If not, then arguably it does not matter that animals are exposed to such events (Bermond 1997). A plant may be dying, but as it has no nervous system to generate mental experiences, the possibility that it might be suffering does not arise. In the present context, we need to establish a view on whether fish are capable of suffering and we approach this by considering the controversial issue of whether they experience physical damage as pain (Rose 2002). To anticipate, our view is that, while fish do not have the neural machinery consciously to suffer pain in exactly the same way that humans do, nor do they have the self awareness or emotionality that some would argue are prerequisites for human suffering, they may well experience some of the adverse states that we associate with pain and emotional distress, thereby compromising their well-being. Our working position in this paper is therefore that if fish are injured or exposed to other harmful conditions, this is a cause for serious concern, not just in terms of responsible stewardship of fish populations (Rose 2002), but also in terms of the welfare of individuals.

People arguing on either side of this debate have used a number of kinds of evidence, none of them entirely satisfactory. For example, one might assume that the longer the life span of a given species of animal and the more sophisticated its general behaviour, the greater its need for complex mental processes such as those that generate the conscious experience of pain. In this context, therefore, it is relevant that the longest-living vertebrates are found among the fishes and that fish behaviour is rich and complicated. For example, we know that some species form mental representations of their environment and use these for quite complex feats of navigation (Reese 1989; Rodriguez *et al.* 1994). Also, many fish live in social groups and some can recognise individual companions (e.g. Swaney *et al.* 2001). Fish can remember negative experiences. For example, paradise fish avoid places where they have experienced a single attack by a predator and continue to do so for many months (Czanyi & Doka 1993) and carp learn to avoid bait for up to three years after they have been hooked just once (Beukema 1970). While animals could show this kind of associative learning without necessarily having conscious awareness (Rose 2002), clearly experiences such as exposure to a predator or tissue damage can be strongly aversive for a fish.

On the specific point of whether fish experience physical injury as pain, it is helpful to consider current knowledge of pain perception pathways in mammals (Figure 1). In this context, the sensory structures that detect harmful (or noxious) stimuli are called *nociceptors* rather than pain receptors, to stress the fact that detecting and responding to noxious stimuli is not necessarily the same as feeling pain (Broom 1998). What do we know of these systems in fishes? As far as the possession of receptors that detect harmful stimuli is concerned, lampreys (very primitive fish) have nerves in the skin that respond physiologically to mechanical pressure, but there is no evidence that these fish show anatomical or behavioural reactions that would accompany nociception (Matthews & Wickelgren, 1978). However, in at least one teleost fish (the rainbow trout), anatomical and electrophysiological examination of the trigeminal nerve (which is known to convey pain information from the head and mouth in higher vertebrates) has identified two types of nociceptor, A-delta and C fibres (Sneddon 2002; Sneddon, Braithwaite & Gentle submitted).

In terms of the machinery that generates the conscious experience of pain in humans, the brain of a fish is clearly far smaller relative to body size (some 300 times smaller by volume) and simpler in structure than that of a human (Kotrschal *et al.*1998). In particular, fish do not have a neocortex, the part of the brain responsible for the subjective experience of pain in humans (shaded pale grey in Figure 2. Rose 2002). However, we know that the same job can be done by different parts of the brain in different kinds of animals. For example, visual stimuli are processed by part of the cerebral cortex in mammals but by the midbrain optic tectum in birds. It is not impossible that parts of the brain other than the cerebral cortex have evolved the capacity for generating negative emotional states/suffering in non-mammalian vertebrates, including fish.

Behavioural experiments are now required to determine whether the nociceptors associated with the trigeminal nerve in fish produce pain-like responses when stimulated with a noxious substance. Such responses would seem likely, given that jawed fish are known to produce some of the natural opiates that are involved in nociception in mammals (Substance P, enkephalins and B-endorphins, Rodriguezmoldez *et al.*

1993, Zaccone *et al.* 1994, Veccini *et al.* 1992, Balm & Pottinger 1995) and that the behavioural response of goldfish to analgesics is similar to that of a rat (Ehrinsing *et al.* 1982). In mammals opiates act at neural levels below the neocortex (Rose 2002), but this does not preclude their having a pain-suppressing effect and one has to ask why they are needed in fish if these animals do not experience pain.

Taken together, these findings suggest that fish have the sense organs and the sensory processing systems required to perceive harmful stimuli and, probably, the central nervous systems necessary to experience at least some of the adverse states that we associate with pain in mammals. Hence our working position that fish have the capacity to perceive painful stimuli and that these are, at least, strongly aversive.

Figure 1. The main components of the pain perception mechanisms in mammals.

Harmful (noxious) stimuli such as chemicals, heat, pressure etc impinge on the animal.

⇩

Sensory neurons for the detection of harmful stimuli (nociceptors) react to specific stimuli. There are two common types of nociceptor: myelinated, small, slow-conducting A-delta fibres and smaller, slower conducting C fibres. These are activated by specific nociceptive stimuli.

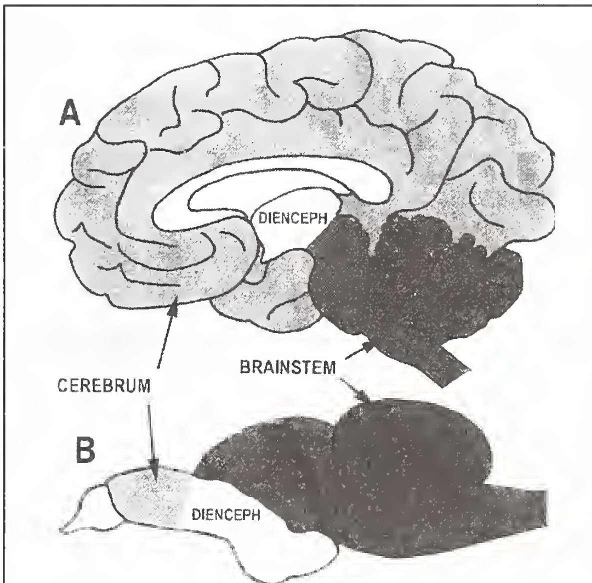
⇩

Activity passes through small nerve fibres in the spinal cord to the thalamus (part of the diencephalon, see Figure 2) and then to the cerebral cortex. There are two partially separate systems, one responsible for the sensory discrimination of harmful stimuli and the other responsible for the conscious, emotional response to such stimuli.

⇩

Physiological responses to harmful stimuli include protective vegetative responses (inflammation and cardiovascular changes) and reflex avoidance responses. More complex, higher order responses include changes in physiology (eg secretion of natural opiates) and behaviour (eg avoidance of locations where harmful stimuli have been experienced).

Figure 2. Comparison of the structure of the mammalian (human) brain (A) and the trout brain (B), in midline view (after Rose 2002). The cerebral cortex is shown in light grey, the brain stem in dark grey.



3. COSTS AND BENEFITS OF HUMAN INTERACTIONS WITH FISHES

Accepting that the welfare of individual fish matters, some would argue that any activity that harms fish should be discontinued. An alternative, utilitarian, view is that an activity is not necessarily wrong because it impairs fish welfare; judgments should be made on both the amount of harm caused to fish and the benefits derived as a result. The Farm Animal Welfare Council 1965) concluded that such a utilitarian approach is appropriate (though only up to a point). With this in mind, Table 1 outlines a number of human activities that may *potentially* compromise the welfare of individual fish and so cause the harm against which any benefits must be weighed. The word "potentially" is stressed because at this point we simply identify areas of possible concern; whether or not any of these activities does harm fish is considered later, in Section 7. Harmful effects on welfare can be indirect, as when we inadvertently alter natural habitats or expose fish to poisonous chemicals, or direct, for example through commercial fisheries, through sports fisheries, through intensive production, through keeping fish as pets or in public aquaria or through scientific research. This briefing paper does not aim to make judgments about what is acceptable and what is unacceptable, but to identify harmful effects of human activities on fish welfare as far as possible, on the basis of current knowledge.

4. NATURAL THREATS TO THE WELFARE OF WILD FISH

Although what is natural is not necessarily good and although there is a clear moral difference between suffering due to natural events and suffering caused by human activity (especially when fish have no choice of environment), an understanding of the threats encountered by wild fish and how frequently these occur (Table 2) can clarify our thoughts on fish welfare. Clearly, wild fish experience injury, poor environmental conditions and stressful events due to encounters with potential predators and with other fish of the same species, to restricted food supplies, to parasitic infection and disease and to natural environmental change. One implication is that fish are likely to have mechanisms for dealing with the adverse conditions that they encounter naturally and that these will come into play during their interactions with humans. These natural responses might provide a means of assessing fish welfare.

5. HOW FISH RESPOND TO NATURAL THREATS TO THEIR WELFARE

5.1 The stress response in fish

Much, though not all, of our understanding of how fish respond to the kinds of natural adverse conditions outlined above comes from the extensive literature on the biology of stress. All animals need a stable internal environment in order to grow, survive and reproduce; the maintenance of this stable environment is termed homeostasis. In response to a destabilizing stimulus (or stressor) the animal seeks to maintain homeostasis through altered behaviour and physiology (the stress responses). The stress response can therefore be considered to be part of an adaptive strategy to cope with a perceived threat to homeostasis (e.g. Sutanto & de Kloet 1994; Tsigos & Chrousos 1994). The stress physiology of fish is directly comparable to that of higher vertebrates (see reviews by Colombo *et al.* 1990; Wedemeyer *et al.* 1990; Barton & Iwama 1991; Wendelaar Bonga 1997), but we know much less about the emotional content of stressors in fish, which are critically important when making the link between physiological stress and suffering (see Section 6).

5.2 Primary stress responses

The immediate neuroendocrine changes that occur when fish are subjected to a stressor (Figure 3) are termed *primary* stress responses, during which perception of the stressor by the fish initiates a rapid, neurally-stimulated release of adrenaline and noradrenaline from the chromaffin tissue, the equivalent of the mammalian adrenal medulla (Gingerich & Drottar 1989). At the same time the hypothalamic-pituitary-interrenal axis is activated (Sumpter 1997) by the release of corticotropin-releasing hormone (CRH) from the hypothalamus and subsequent release of cortisol from the interrenal tissue, the equivalent of the mammalian adrenal cortex (Okawara *et al.* 1992; Weld *et al.* 1987).

Table 1. Some human activities that may *potentially* compromise fish welfare, and a reminder of the benefits these can confer. Evidence on whether such activities actually do or do not compromise welfare is given in Section 8.

ACTIVITY	EXAMPLES OF *POTENTIAL* EFFECTS ON WELFARE	EXAMPLES OF POSSIBLE BENEFITS
Environmental degradation	• Reduced availability of natural food • Introduction of exotic species into existing fish communities • Habitat modification, creating (e.g.) sub-optimal temperatures or flow regimes • Loss of or displacement from natural habitats • Reduced population densities (or crowding) and abnormal social experiences • Disturbance through tourism • Acute and chronic exposure to pollutants and litter	There are numerous examples of cases where humans have benefited from activities that alter the natural habitat of wild fish. e.g. Dams provide humans with power, irrigation, flood control and recreation, but result in loss of habitat (including spawning habitat) for fish and barriers to migration (International Commission on Large Dams http://genepi.louis-jean.com/cigb/BandC.pdf)
Commercial and sports fisheries	• In both, tissue damage, physical exhaustion and severe oxygen deficit during capture • In both, pain and stress during slaughter • In angling, pain and stress in tethered fish when live bait is used • In angling, release of reared fish inappropriately equipped for survival in the wild • In angling, stocked fish introduced to lakes may be denied the opportunity to migrate	In 1995 there were 20 million anglers in the USA, creating 1.3 million jobs and with an estimated value of 70 billion US dollars. (USA Fish and Wildlife Service Survey)
Intensive production	• High densities in simple and constraining conditions, both in normal rearing conditions and for husbandry • Poor water quality • Aggressive interactions, which can cause damage and constrain access to food • Food deprivation (e.g. during disease treatment and before harvest) • Handling and removal from water during routine husbandry procedures • Unnatural light-dark regimes, to suppress breeding • Handling, constraint and, sometimes, low oxygen levels during transportation • Permanent adverse physical states and possibly increased levels of aggressiveness due to selection for fast growth • Increased exposure to predators, attracted to fish farms or used to grade out smaller fish (in Tilapia aquaculture) • Transmission of disease to wild stocks • Crowding, handling, removal from water and pain during slaughter	Between 1987 and 1997 world production of farmed fish increased from 10.6 to 28.3 million tonnes (FAO, 1998). 75% of the rural population in some areas of Vietnam is engaged in aquaculture. Even in 1985, the European aquaculture employed 150,000 people (Ackefors 1989)
Keeping ornamental fish and display fish in public aquaria	• For ornamental fish, capture by sub-lethal poisoning • For ornamental fish, permanent adverse physical states due to selective breeding • For ornamental fish, release or escape of exotic species • Inappropriate temperatures, poor water quality and physical constraint during transport • Confined space and poor water quality once housed. • Inappropriate physical conditions • Inappropriate social conditions, with shoaling fish at low densities and predators with prey • Inappropriate diets	Ornamental fish are the third most popular pet after dogs and cats, up to 40 million entering the USA and up to 1 million entering the UK per annum. The retail value of the fish and accessories ranged between 189 and 305 million US$ (Andrews 1990, Mintel 1991). Sustainable keeping of endangered species has conservation value
Scientific research	• Fish used in the laboratory for experimental purposes are often confined and may be exposed to a range of deliberately-imposed adverse physical, physiological and behavioural states • Fisheries research often involves electrofishing, tagging, fin clipping or otherwise marking fish, which potentially cause pain and injury. • In both laboratory and field, handling during research procedures may cause injury	In addition to underpinning effective aquaculture and aquarism, scientific research on fish can have benefits for human health. e.g. Toxicology testing (Purchase 1999) and biomedical research using the pufferfish model genome (Hedges & Kumar 2002). Where research leads to (e.g.) better husbandry procedures, fish themselves benefit

Table 2. Natural threats to the welfare of wild fish	
STRESSOR	**COMMENT AND EXAMPLES**
Predators	• Predation rates can be very high. e.g. Excluding predators reduces mortality by 36% in wrasse (Shima 2002) • Unsuccessful predatory attacks may cause wounding and an increased risk of disease. e.g. 30% of wild sticklebacks bear injuries due to failed predatory attacks (Reimchen 1994) • The threat of predation may suppress feeding and may cause fish to forage sub-optimally (Hart 1997)
Conspecifics	• Many species live naturally in groups of the same species, which provide protection against predators. Obligate shoaling fish separated from companions will strive to join a shoal (Pitcher and Parrish 1993). • In many other species (or in shoaling species under particular circumstances) conspecifics fight over resources and this can cause physical damage and depletion of energy reserves (Neat *et al.*1998). Many wild Atlantic salmon that mature as juveniles have wounds from attacks by larger males (Garcia de Leaniz 1990). Losers may be deprived of resources and/or exposed to chronic social stress (Abbott and Dill 1989, Alanara 1997)
Food availability and body condition	• Wild fish often experience periods of when food is in short supply (Dutil and Lambert 2000), though many species have flexible metabolic systems to cope with periods of prolonged food deprivation (O'Connor *et al.*2000a) • Growth rates in fish held captive with excess food consistently and markedly exceed those achieved by fish in the wild (e.g. in *Cynolebias viarius* – Errea & Danulat 2001) • Lipid deposition rates and mineral content of body tissues may also differ between wild and captive reared fish (e.g. Orban *et al.*2002 for sea bass) • Many larvae (at least 50% in the common Japanese goby *Rhinogobius brunneus*) die through starvation prior to obtaining their first food (Iguchi & Mizuno 1999)
Extensive migration	• Daily vertical migration by pelagic fishes results in slower growth (Lima 1998) • Energy reserves in spawning salmon are reduced by more than 90% following upriver migration (e.g. Johnson *et al.* 1991)
Parasites and disease	• In the wild most fish carry a parasite burden that impairs their health • High gill parasite loads in fish from the Slaton Sea, California, cause damage to gills, poor respiration and osmoregulation and juvenile mortality in several species (Kuperman *et al.*2001) • In Orange roughy *Hopostethus atlanticus* from New Zealand, parasite loading is negatively correlated with growth (Gauldie & Jones 2000).
Suboptimal environmental conditions	• Most environmental variables fluctuate naturally, so wild fish will experience conditions that deviate from optimal for the species concerned. • Fish can avoid or adapt to sub-optimal environmental conditions (at an energetic cost), but exposure to conditions beyond their limit of tolerance is, by definition, lethal

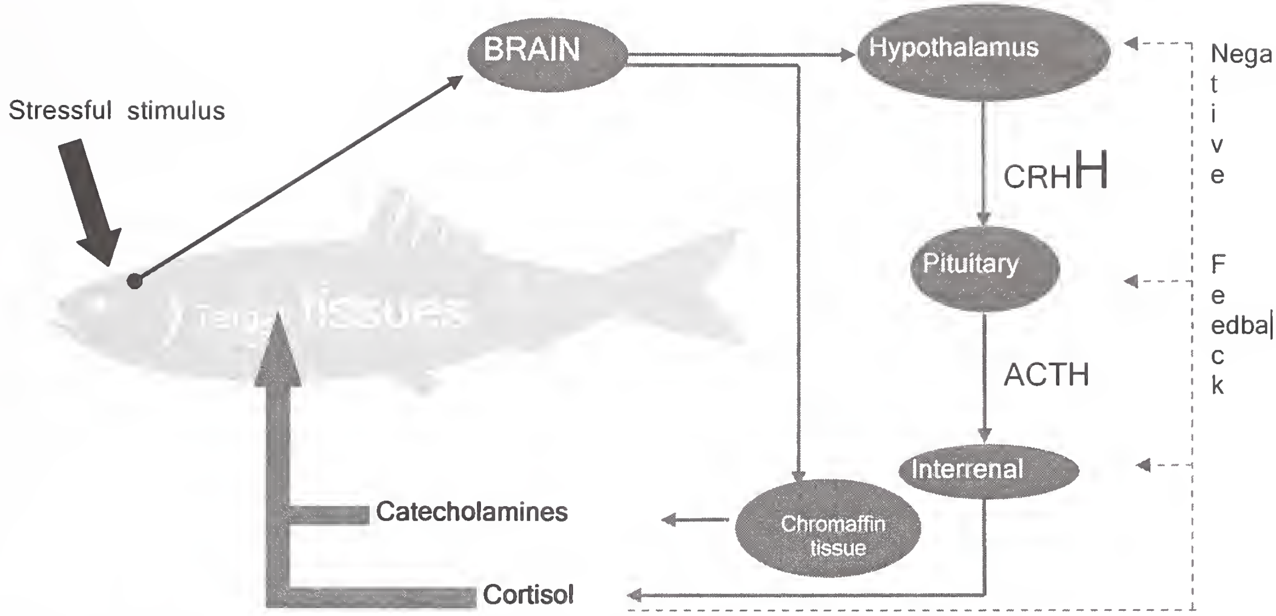


Figure 3. The main hormonal components of the stress response in fishes.

Because of the rapidity of the catecholamine response following disturbance and consequent difficulties in accurately measuring blood levels in free-swimming fish, we know little about the dynamics of the catecholamine response to stressors in fish, other than that it is extremely rapid. As far as cortisol is concerned, the size and duration of stress-induced elevation in plasma levels are usually proportional to the severity and duration of the stressor. Recovery from short-term, acute stress takes a matter of hours (Pickering & Pottinger 1989, Waring *et al.* 1992), but elevated cortisol levels generally persist during continuous, chronic stress (Pottinger & Moran 1993, Pottinger *et al.*1994a). Under some circumstances, fish will acclimate to a repeated stressor and cease to show a stress response, despite initially responding with elevated cortisol levels (Pickering & Pottinger 1985).

5.3 Secondary stress responses

As a direct consequence of elevated circulating levels of catecholamines and cortisol, a wide range of *secondary* changes are evoked. These include:

- Altered rates of secretion of other pituitary hormones and of thyroid hormones.
- Changes in rates of turnover of brain neurotransmitters such as dopamine and serotonin.
- Improved respiratory capacity via increased heart rate and stroke volume and increased blood flow to the gills (at the expense of disrupted salt and water balance).
- Mobilization of energy by breakdown of carbohydrate and lipid reserves and by oxidation of muscle protein.

5.4 Behavioural responses to stress

In some respects, behavioural responses are an animal's first line of defence against adverse environmental change, often being triggered by the same stimuli that initiate the primary stress response. The exact behavioural response depends on the stressor concerned. For example, after an attack by another fish of the same species, fish may flee and hide or take up a submissive posture, often with altered body colour (e.g. O'Connor *et al.* 2000). When attacked by a predator, fish may respond by shoaling (Pitcher & Parrish 1993), "freezing" (e.g. Goodey & Liley 1985) or taking shelter (e.g. Brown & Warburton 1999) and may change colour in this context as well (Endler 1986). Feeding may be suppressed following an encounter with a predator, or inefficient feeding strategies may be adopted (Hart 1997) and fish may avoid areas in which they have been attacked (Lima 1998). Specific adaptive behaviour patterns are observed in response to tissue damage (for example, fish that are hooked in the mouth show rapid darting, spitting and shaking of the head, Verheijen & Buwalda 1988) or to parasitic disease (Furevik *et al.*1993).

5.5 Tertiary stress responses

The primary and secondary stress responses are short-term effects of acute, short-lived challenges. Where a stress response is prolonged or repeated (Schreck 2000) and the fish has no way of avoiding or escaping the challenge, a series of *tertiary* effects become apparent, including changes in immune function and disease resistance, in growth and in reproductive status.

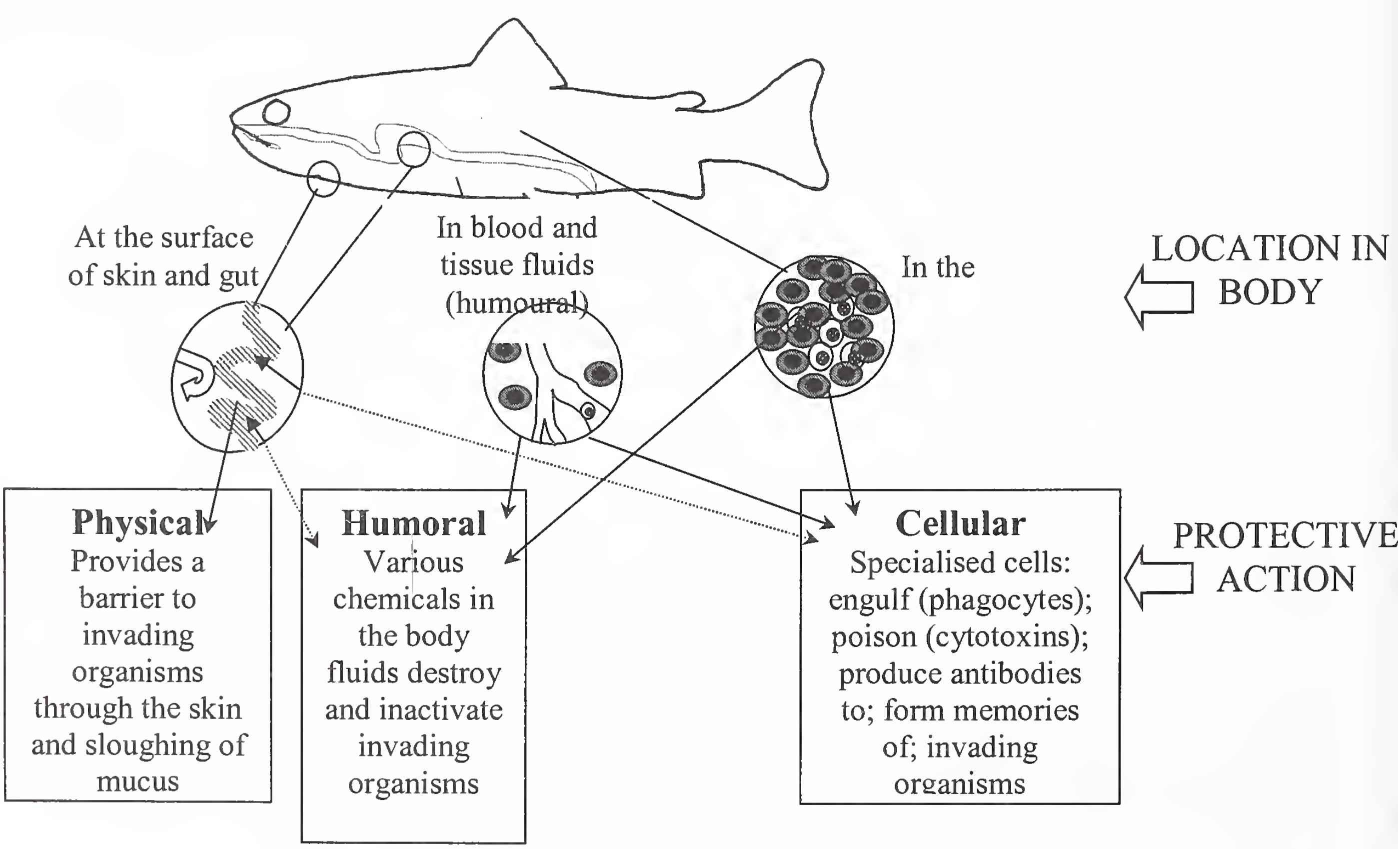
5.5.1 Changes in immune function and disease resistance

Animals are defended against invasion by disease-causing organisms by their anatomy, physiology and normal microflora (Bøgwald *et al.* 1994, Ringø & Gatesoupe 1998). Jawed fish all have a non-specific immune system that does not depend on prior disease challenge and also a specific immune system with a memory component that can adapt to different invading organisms (Flajnik 1996, Klein 1997, Warr 1997; Press 1998). Compared with higher vertebrates fish are more reliant on the non-specific immune system and the specific system is less well developed. The main components of these systems (summarized in Figure 4) are:

- Chemicals in the body fluids that are able to destroy or inactivate invading organisms.
- Circulating and tissue-dwelling cells that engulf or destroy invading organisms (phagocytes).
- In the specific system, circulating cells responsible for antibody production (lymphocytes) and phagocytic cells, which have an additional role in presenting antigens to the specific immune system.

As in mammals, the best known link between stress and immune status in fish arises through effects of cortisol (Wendelaar Bonga 1997). Cells involved in the immune response contain receptors for cortisol (Maule & Shreck 1990) and raised cortisol concentrations suppress many aspects of immune function. However, the relationship between stress and the immune system goes in two directions, since components of the immune system can influence stress responses (Ottaviani & Franceschi 1996, Balm 1997). For example, cytokines (chemicals that are secreted by blood cells - leukocytes including macrophages - in response to a microbial challenge) can cross the blood-brain barrier and affect secretion of stress mediators and stress hormones. Additionally, some cells of the immune system (macrophages) are known to produce stress mediators such as ACTH (Brown 1994).

Figure 4. The components of the immune response in fishes. The different elements interact and communicate to protect fish from invasion.



One consequence of such stress-induced changes in immune function is that chronic exposure to adverse conditions makes fish more vulnerable to disease. Following administration of cortisol to salmonid fish, mortality due to fungal and bacterial pathogens increases (Pickering & Pottinger 1989) and there are numerous reports of stress-mediated bacterial diseases, typical examples include furunculosis and vibriosis (Plumb 1994).

5.5.2 Changes in growth and reproduction

Growth in most fish is indeterminate and flexible and varies over short time scales as rates of energy intake and utilization change. Many of the effects of stress described above cause reduced energy intake and increased energy utilization, so stress is likely to reduce rates of growth indirectly through a negative effect on energy balance. In addition, growth hormone secretion is reduced in fish during periods of stress (Pickering *et al.* 1991, Farbridge & Leatherland 1992), so there are also direct effects on the mechanisms that control growth. A number of studies have demonstrated reduced growth in response to administration of cortisol (Barton *et al.* 1987) and to prolonged activation or frequent intermittent activation of the stress response in the laboratory (e.g. Pickering 1993). Poor growth has been reported in wild fish populations as a result of environmental stressors such as altered pH (e.g. Puste & Das 2001), reduced dissolved oxygen levels (Kramer 1987) and altered salinity (Brett 1979).

Growth and reproduction are complementary processes that depend upon availability of energy and nutrients obtained through foraging. Life history strategies have evolved to produce patterns of growth that maximize lifetime reproductive capacity, in a condition-dependent manner that takes nutritional status into account (Thorpe *et al.* 1998). Since growth and reproduction are adaptively linked, stress-induced impairment of growth may indirectly interfere with maturation. Additionally, reproductive activity can be suppressed directly during periods of stress, via an effect on reproductive hormones (Pickering *et al.* 1987, Donaldson 1990, Carragher & Pankhurst 1991, Campbell *et al.* 1992, Pankhurst & Dedual 1994, Pankhurst & Van der Kraak 1997).

5.6 Functional considerations

Most natural stressors are probably short lived, since fish will either avoid/overcome the challenge or succumb. The beneficial nature of the secondary stress responses to such short-term stressors is evident - all are associated with maintaining cardio-respiratory performance and mobilising energy under challenging conditions (Randall & Perry, 1992). Some tertiary responses also may be adaptations to adverse conditions; for example, stress-induced suppression of immune function may prevent auto-immune lesions and suppression of reproductive activity may be an adaptive "switching-off" of an energy demanding process in poor environmental conditions. Other tertiary responses (e.g. diseases arising from weakened defences) are wholly adverse in nature.

6. ASSESSING FISH WELFARE

6.1 Ways of measuring fish welfare.

This knowledge of the natural responses of fish to adverse conditions suggests ways of probing the physical, physiological or psychological state of individual fish to determine whether their welfare is compromised. The aspects of an animal's condition that are often used in this context are its health status, its physiology and its behaviour. Relating each of these to welfare is not simple, and ideally all three should be taken into account when assessing welfare in any given case.

6.1.1. Stress physiology and fish welfare

Stress responses represent an animal's natural reaction to challenging conditions and are often used as indicators of impaired welfare, so studies of physiological stress feature prominently in welfare research. However, it is important to recognise that physiological stress is not synonymous with suffering (Dawkins 1998). Low cortisol levels might mean that a fish is not stressed, but it might mean that the capacity of its interrenal tissue to produce cortisol is exhausted.

There is no particular reason to suggest that the temporary physiological activation that prepares fish for activity is detrimental to welfare and in some contexts short term stress responses (for example, in anticipation of feeding) may well be beneficial (Moberg 1999). Tertiary stress effects such as suppressed reproduction may well be adaptive responses to poor condition in the wild, but even so it seems reasonable to assume that in captive fish they indicate exposure to chronic, unavoidable stress, which may compromise welfare. Thus although the concept of stress does not fully capture the complexities of animal welfare, monitoring stress responses may give us part of the picture. In particular, where several components of the stress response are all influenced in a similar way by the same condition, this suggests that there is cause for concern about the welfare of the fish involved.

6.1.2 Health, disease and welfare

The link between health and welfare is complex. If an individual fish shows signs of disease, it seems reasonable to infer that it is in a poor state of welfare, for that very reason. The converse is not necessarily true, because the welfare of a healthy fish may be compromised in other ways, for example if a schooling fish is held in isolation. In addition to welfare problems raised directly by the signs of disease themselves, because stress can suppress immune function, a high incidence of disease (and mortality) is often taken as a warning sign for other welfare problems. The causes of disease in fish are invariably complex and the risk of disease does indeed increase as the conditions under which the fish are living deteriorate, in the wild or in captivity. Therefore the occurrence of disease in fish will usually be associated with a poor state of welfare and may be an indication that there is an underlying problem with the environment or conditions to which the fish are subjected. However, it is overly simplistic to assume that disease is invariably the result of poor living conditions; even fish experiencing optimal conditions may suffer from disease and will, eventually die. Nor does the occurrence of disease inevitably imply that the problem is due to human mismanagement and that it is possible to rectify the situation. Infectious diseases occur and cause significant losses in populations of wild fish and there are well-documented examples of serious epidemics in wild populations (e.g. Epizootic Ulcerative Syndrome, Lillie *et al.* 1996). Under some circumstances disease may be preventable in captive populations, for example where some form of therapy or vaccine exists, and this may promote fish welfare.

6.1.3 Behaviour and welfare

The idea that animals suffer if they cannot perform their full behavioural repertoire has been used to identify conditions that detract from welfare (Section 1.3), so behavioural studies have an important role in welfare research (Mench & Mason 1997). In addition, because altered behaviour is an early and easily observed response to adverse conditions, specific responses to natural stressors (such as 'freezing' in the presence of a predator or chafing/flashing to remove ectoparasites) can be used as an indicator of impaired welfare. Finally, and importantly since so many controversial issues in discussions of welfare stem from the impossibility of getting inside the minds of animals, choice tests that allow animals to express their natural preferences can help to identify things that may promote/detract from their welfare. This approach has proved useful in welfare research on birds and mammals, even though the underlying assumption that animals choose what is good for them is not always valid (Dawkins 1998).

There are many examples of the use of choice tests in fish (for example, choice between different temperatures or between schools of different sizes). The results have not usually been directly related to fish welfare, but can be interpreted in this context. For example, fish of various species avoid potentially lethal concentrations of some harmful pollutants such as copper, suggesting that these impair welfare; however, they ignore other equally harmful substances such as selenium (Giattina & Garton 1983), which either means that these do not impair welfare or that fish are unable to detect them and so cannot choose what is good for them. Similarly, territorial damselfish will learn to swim through a simple maze if rewarded by the opportunity to display aggressively to a neighbouring damselfish (e.g. Rasa 1971). This suggests that being involved in an exchange of aggressive displays (as opposed to losing or being injured) is not necessarily aversive and so may not impair welfare, at least when the fish concerned can chose whether to interact with its neighbour.

6.2 Sensitive and easily applied welfare indicators for fish

Collecting data on fish physiology, biochemistry and behaviour is time consuming and technically complex. It also involves handling and anaesthetizing or killing fish in order to collect blood or other tissue. There are non-invasive methods such as measuring cortisol levels in the water in which fish have lived (Oliviera 2001, Scott *et al.*2001), but some of these lack the precision of measurements made on individual fish. Such intensive work is necessary in scientific research, but is impractical for everyday use, in pet shops or on working fish farms for example. What is needed here is a set of simple, non-intrusive signs or danger signals that can be used easily without needing access to laboratory apparatus. A number of possible hands-off welfare indicators can and have been used to assess the welfare of individual

fish and these are listed below. All are well known to people with a practical interest in fish welfare, such as good fish farm managers and careful owners of ornamental fish.

Indicators

Changes in colour: Stress-induced changes in skin or eye colour (with a complex hormonal background) have been reported in a number of fish species, including ornamental species (Etscheidt 1992), and so could be a sign of exposure to adverse events. Eye colour as an index of social stress/subordinate status in salmonids provides an example.

Changes in ventilation rate: A high oxygen demand is reflected by rapid irrigation of the gills. The rate of opercular beats is therefore increased by stress and can be counted, automatically or by eye. This, together with a visual assessment of gill status, is used as a sign of incipient problems in ornamental fish (Etscheidt 1992) and to monitor exposure to pollutants in salmonid fish.

Changes in swimming and other behaviour patterns: Fish may respond to unfavourable conditions by adopting different speeds of swimming and by using of different regions of a tank or cage (Morton 1990, Etscheidt 1992, Juell 1995). Abnormal swimming has been used as a sign of poor welfare in farmed fish (Holm *et al.* 1998). Known behavioural responses to adverse events and conditions are potential signs of both general and specific trouble (Morton 1990). These include excessive activity or immobility (Etscheidt 1992), body positions that protect injured fins, escape attempts in confined conditions and chafing movements to dislodge ectoparasites (Furevik *et al.*1993).

Reduced food intake: Notwithstanding that there are many reasons why a fish might not eat, the fact that feeding is suppressed by acute and chronic stress means that loss of appetite is potentially a sign of impaired welfare.

Slow growth: Notwithstanding that growth rates in fish are flexible and naturally variable, sustained reductions in growth may be indicative of chronic stress. Thus where fish are regularly weighed or where size can be assessed by eye (or by underwater camera) slow growth can be used as a possible sign of trouble.

Loss of condition: Fish change shape and/or lose weight for many reasons, but because reduced feeding and mobilisation of reserves are secondary stress responses, where fish are regularly weighed and measured, or where body shape can be assessed by eye (for example by the visibility of the vertebrae, Escheidt 1992) loss of condition can be used as a possible sign of trouble.

Morphological abnormalities: Because adverse conditions can interfere with normal development, the occurrence of morphological abnormalities can be used as an indicator of poor larval rearing conditions (Boglione *et al.* 2001).

Injury: Injury may be a direct consequence of an adverse event, in which case, the presence of such injuries is a sign of poor welfare. For example, dorsal fin injury in salmonids is often caused by attacks from conspecifics (Turnbull *et al.* 1998) and scales that are dislodged rather than lying flat are a sign of poor welfare in ornamental fish (Etscheidt 1992). In addition, because immune responses can be suppressed by cortisol, slow recovery from injury (or a high incidence of injury) may be a sign of generally poor conditions. However, fin erosion has multiple causes and these are not fully understood

Disease states: Since the causes of most aquatic diseases are complex and dependent on environmental conditions, a diseased state can indicate an underlying problem with the environment or management. Increased incidence of disease in any population of fish should be treated as a warning that there may be other underlying problems. However, interpreting the welfare implications of an observed disease requires a detailed understanding of the natural history of the disease and in some cases diseases are not sufficiently well understood to interpret their implications for welfare.

Reduced reproductive performance: For many farmed species, reproduction is prevented or avoided in growing stock. Where this is not the case, for example, in brood stock or where ornamental fish are concerned, because chronic stress impairs reproductive function, failure of adult fish to breed or to display normal patterns of reproductive development when feed, light and temperature regimes are appropriate is a possible sign of poor welfare.

How well these signs work in any given case will depend on the species concerned (eye colour may be a good indicator of social stress in salmon, but not in sticklebacks), on circumstances (depleted energy reserves might be cause for concern in an immature salmon, but not in one that has just bred) and also on individual status (failure to feed may be a sign of poor welfare in a juvenile salmon in the summer, but not necessarily in the winter when they may show adaptive natural anorexia). In addition, the potential for using this full list will vary with the context in which fish welfare is to be assessed; fish farmers may have to rely on a few signs, collected on a small proportion of their stock, but people keeping ornamental fish are well placed to use many of them.

7. HOW DO HUMAN ACTIVITIES AFFECT FISH WELFARE?

The scientific study of fish welfare lags behind that of the welfare of other vertebrates (reflecting the pressure of public concern), but there is still an extensive literature on the subject, using the welfare indicators outlined above. A survey of this literature shows that different studies do not always come up with the same results. This is not necessarily because any of them are wrong. Rather it reflects the complex nature of fish welfare and the fact that the various factors that impact on it may interact. For example, a given stocking density may generate signs of poor welfare at one temperature or level of disturbance, but not at another and conditions appropriate for one species may be entirely inappropriate for another. We are not yet in a position to make definitive statements about how a given activity impacts on fish welfare and more research is needed.

However, people reading this Briefing Paper will wish to know how particular human activities affect fish welfare, so this section gives a brief synopsis of current understanding on the subject (Table 3), using just a few examples. We concentrate on three activities, chosen because they are of known current public concern and because people can have an impact on these activities through their own personal choices. These are: the keeping of ornamental fish (one can choose not to keep ornamental fish or buy only from suppliers who take welfare seriously), angling (one can choose not to go fishing or to use practices that minimise suffering) and aquaculture (one can choose not to eat farmed fish or to buy only from sources that guarantee a high standard of welfare). On this last point, it should be noted that the welfare of fish caught by commercial fisheries (still the largest area of human-fish interactions) is also a cause for serious concern. Fish are harmed by capture (e.g. cortisol levels increase in sea bream captured by trammel net and many fish are mortally injured, Chopin & Arimoto 1995) and slaughter methods (especially asphyxia) are highly stressful (Poli *et al.* 2002). In addition, non-target species captured as by-catch are often injured or killed (Pronovi *et al.* 2001). Scientific research on fish raises serious ethical concerns, but is already strongly regulated in many countries to ensure that harm in terms of compromised welfare is outweighed by benefits in terms of enhanced knowledge on important issues (e.g., the UK Animals (Scientific Procedures) Act, 1986). As far as environmental degradation is concerned, while this is clearly a cause of poor welfare in very large numbers of fish (Montgomery & Needleman 1997), we have many more pressing motives for minimising human impact on the environment than concern for fish welfare.

A number of general points emerge from this synopsis:

1. *What we do to fish does indeed compromise their welfare:* If one accepts chronically elevated cortisol levels, impaired disease resistance and poor growth (for example) as indicators of welfare in fish, then Table 3 gives many examples of harmful effects of human activities on fish welfare.

2. *Fish are different from other vertebrates in ways that have important implications for welfare:* The effects of human activity on fish welfare are not always what one would predict by extrapolating from birds and mammals. For example, fish allow their body temperature to fluctuate with that of the environment (i.e. they are ectothermic animals) and also show striking natural variation in appetite and the evidence suggests that food deprivation is not such a critical aspect of their welfare; this is not to say that it is acceptable to starve fish for long periods but that under appropriate circumstances periods of food deprivation may not cause welfare problems. Similarly, for species of fish that naturally spend their lives in dense shoals with many companions, being held at too low rather than too high a density may cause welfare problems. On the other hand, fish are in intimate contact with their environment through the huge surface of their gills and current evidence shows that many aspects of water quality are critical for their health and welfare.

3. *It is not possible to specify conditions that guarantee fish welfare:* The effects of varying one factor (such as density) frequently depends on the status of other factors (such as disturbance and water quality), which highlights the fact that, even for a particular species, gender and age of fish we cannot guarantee the welfare by defining a simple set of husbandry conditions. This in turn emphasises the need for sensitive on-the-spot indicators of welfare.

8. ANOTHER LOOK AT CRITERIA FOR FISH WELFARE

Knowing how fish respond to adverse conditions, we can revisit the question of what constitutes fish welfare, reworking the five welfare domains specified by Mellor & Stafford (2001) (and used in the RSPCA's report on standards for farmed Atlantic salmon, 2002) into a form that is more appropriate for fish. The details under each domain will depend on the species concerned, and also on age, gender and reproductive condition.

Domain 1. Water and food deprivation, malnutrition

Fish should:

- Have access to an adequate, nutritionally complete diet, taking into account the fact that fish vary greatly in their natural diet, that they do not need to maintain a constant body temperature and that in many cases they show marked differences in requirements with season and life history stage.
- Be presented with food in a manner that is appropriate to the natural feeding behaviour of the species concerned (e.g. pellets of the correct shape to elicit feeding) and that avoids undue competition.

The natural diet of wild fish varies markedly between species and as with other vertebrate groups, it is important to ensure that captive fish are given a nutritionally appropriate diet, although in many cases we do not know exactly what this should be. For example, diets lacking in critical micronutrients impair welfare in many species, according to a range of indicators, such as high mortality, morphological abnormalities, poor immune function, abnormal behaviour, poor feeding, impaired sensory function and slow growth (De Silva & Anderson 1995).

The fact that fish are ectotherms is critical to how they are affected by periods of food deprivation. They do not need to maintain a fixed body temperature, so periods without food are less detrimental than for endothermic animals (that must do so). This is not to say that it is a matter of indifference if fish are starved; they certainly have mechanisms that motivate them to feed when their stomachs are empty and their nutritional reserves are low and restricted food may have other effects such as increasing levels of aggression. However, it does mean we cannot extrapolate from what we know about the effects of food deprivation in birds and mammals when considering appropriate conditions for fish. Expressing periods of deprivation in "degree days" takes this complexity into account. Secondly, wild fish show marked changes in appetite (some temperature-based and other depending on life history events) that determine the effect of food deprivation on welfare. In the winter juvenile salmon may become naturally anorexic, eating little for weeks (Metcalfe *et al.*1988). These fish will feed when their energy reserves fall to a critical level, but up to this point, low rations would not compromise welfare. On the other hand, maturing salmon show a spontaneous peak in appetite in spring, when nutrient reserves for migration and spawning are accumulated, and food deprivation at this point may well compromise welfare (Kadri *et al.*1993).

Domain 2. Environmental challenge

Critical for fish welfare are:

- Water quality, flow rates and temperature appropriate for the species concerned
- Appropriate seasonal and daily patterns of light intensity
- Provision of cover and shelter

Fish are in intimate contact with their environment through the huge surface of their gills and of necessity they defecate into the medium in which they live, so water quality (in terms of dissolved oxygen, ammonia and pH) and the presence of contaminants (organic and inorganic pollutants) are probably the most critical aspects of the environment for fish welfare and also the best defined. Optimal conditions vary markedly

between species; for example, catfish do poorly in clear water, whereas salmon do poorly in cloudy water and cyprinid fish are very tolerant of low dissolved oxygen levels whereas salmonid fish are not. The flow characteristics of the fish's natural habitat are also of importance, some species preferring static water, others tolerating or preferring relatively high flow rates. The nature of the substratum is also important for welfare, particularly in bottom-dwelling species; skin diseases are less common in flat fish housed in tanks with rough rather than smooth bases.

Domain 3. Disease, injury and functional impairment

Disease should be prevented or rapidly diagnosed and treated where possible.

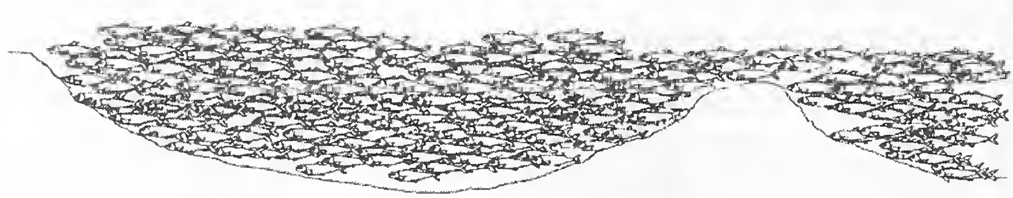
Diseases frequently indicate an underlying environmental problem, so diagnosing and controlling a disease must always take account of the whole system and not consider the fish alone. Diseases of fish are mostly species and system specific and many are poorly understood.

Domain 4. Behavioural/interactive restriction

- Fish should have sufficient space to allow a degree of freedom of movement, but the definition of 'sufficient' will be species-specific.
- For shoaling species, the company of their own kind is important for welfare, but for territorial species, this may not be the case.
- A degree of environmental complexity may be important, depending on the species concerned

Many species form dense schools in the wild (Figure 5) and this is important when assessing the welfare if such species are held at high density, so here too it can be misleading to extrapolate from birds and mammals to fish. As discussed in Section 1.3, we do not know whether fish such as salmon are motivated to migrate by a particular route (as opposed to swimming long distances, which they can do in farm cages for example); if this were the case, their behavioural needs could not be met in a sea cage. The concept of "facilities" (Mellor & Stafford 2001, see section 1.4) may be inappropriate for fish, though some species need shelter or cover, some may require nesting material when breeding, some need tough structures on which to chew (Etscheidt 1995).

Figure 5. Schematic representation of a natural shoal of herring, based on echosounding (Mackinson 1999)



Domain 5. Mental and physical suffering

Conditions that produce unacceptable levels of anxiety, fear, distress, boredom, sickness, pain, thirst, hunger and so on should be minimised in fish as in other vertebrates, but we know little about such states in fish, or how to recognise them.

Domain 5 is critical since it relates adverse experience to emotional response. The subjective experience of fish are very hard to understand, so it is not easy to identify "conditions that produce unacceptable levels of anxiety… and so on." Most of the cues that are employed to identify fear and distress in other vertebrates are simply not accessible for fish - for example, there are no direct parallels for facial or vocal signalling. Greater understanding of cognitive processes in fish is needed before we can make the link between welfare and suffering in this group.

9. AREAS OF IGNORANCE

Clearly, a fair amount of information is available about fish welfare and about how common human practices impinge on it, but equally clearly there are big gaps in our knowledge. Some of these areas of ignorance concern issues that are fundamental to the whole concept of fish welfare, what it means and how it might be measured. These are critical areas where better general understanding is needed if we are effectively to promote fish welfare, though providing this better understanding will certainly be challenging.

- The single most important area of ignorance is a lack of understanding of the mental capabilities of fish and whether and how measurable things (such as physical damage and the physiological and behavioural responses to challenge) generate subjective states of well-being or suffering.
- On a related point, there is an equally pressing need for better understanding of the behavioural responses of fish to noxious stimuli and the neural mechanisms (from sense organs to higher brain function) that produce these responses.
- Also on a specific point, for each exploited species, it is important to discover whether there are actions that the fish are highly motivated to perform and that, like nest building in domestic hens, may be described as 'behavioural needs'.
- More knowledge is required about diseases in fish, about the links between stress, immune function and disease states and therefore about the relationship between health and welfare.
- In practical terms, a better array of welfare indicators (for example, easily observed morphological and behavioural cues) is requires for everyday use in circumstances where time consuming scrutiny of fish is impossible. In this context, more information is needed on the causes of fin erosion.

Other gaps in existing knowledge are also important, but will be somewhat easier to fill because they involve expanding the information already available for some species and in some contexts.

- A certain amount is known about the effect of angling and aquaculture practices on fish welfare, but there is very little information on the welfare of ornamental fish, particularly from capture to point of sale. Questions also remain about conditions within aquaria and ornamental ponds - what are the effects of being confined in a small, exposed space, of social isolation or of frequent interactions with a predator?
- Where angling and aquaculture are concerned, much is known about effects on welfare of salmonids, but little information is available about other kinds of fish that are reared commercially or caught by anglers. For example, information is needed on the effects of food deprivation and of different methods of slaughter in more kinds of fish.
- Even for the well studied species and well documented effects, the exact mechanism by which the adverse effects come about are unknown. For example, there is plenty of evidence of poor welfare in salmon and trout held at very high densities, but it is not clear whether this is the result of poor water quality, high levels of aggression, simple physical damage or some other process (Ellis *et al.* 2002). The effects of density must be separated from those of other factors before appropriate remedial measures can be developed.

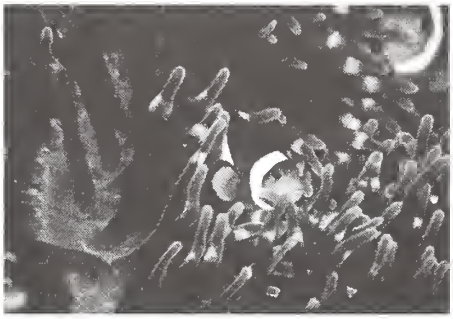
10. CONCLUSIONS

By spelling out current understanding on the welfare of fish, this Briefing Paper will hopefully contribute to debate on the subject. This is a difficult area to review, because many academic disciplines have an interest in it, because complex concepts are involved that are hard to define and because there are large areas of ignorance and, consequently, of disagreement. In this document a pragmatic working position has been taken on a number of important questions (Do fish suffer? Does this matter?), recognising that this position may have to be changed in the light of facts that emerge in the future. In spite of these difficulties, a great deal of painstaking research has shown how fish respond to the adverse events that they experience in nature and how these could be used to probe their welfare. A picture is beginning to emerge (partial and blurred at present) of how various human activities impinge on the welfare of fish and therefore of what might be done to improve matters. This review of the literature on fish welfare highlights the need for better knowledge and a fuller understanding of fish welfare, which will allow the development of better ways of promoting it. In the shorter term, the broader aim of improving the welfare of fish might best be achieved by education - by demonstrating that fish are very sophisticated animals in all respects and are far removed from unfeeling creatures with a 15 second memory of popular misconception. A heightened appreciation of fish by those who work with or exploit them might go a long way towards redressing some current shortcomings in fish welfare.

Table 3. Overview of current scientific understanding of the impact of common practice in, aquaculture, angling and the keeping of ornamental fish, with a few representative examples.	
AQUACULTURE	**SOME DEMONSTRATED EFFECTS ON WELFARE**
Transportation	Certain kinds of transportation induce physiological stress responses and a prolonged recovery period may be necessary (Bandeen & Leatherland 1997, Barton 2000, Rouger *et al.* 1998, Iversen *et al.* 1998, Sandodden *et al.* 2001).
Handling/netting	Physical disturbance evokes physiological stress responses in many species of farmed fish (reviewed by Pickering 1998) and reduces disease resistance (Stangeland *et al.* 1996).
Confinement and short-term crowding	Physical confinement in otherwise favourable conditions increases cortisol and glucose levels and alters macrophage activity in various species (Garci-Garbi *et al.* 1998). Carp show a mild, physiological stress response to crowding that declined as the fish adapted, but crowded fish are more sensitive to an additional acute stressor (confinement in a net; Ruane *et al.* 2002). Crowding during grading increases cortisol levels for up to 48h (Barnett & Pankhurst 1998).
Inappropriate densities	High densities impair welfare in some species (trout, salmon: Ewing & Ewing 1995, bass: Vazzana 2002, red porgy: Rotllani & Tori 1997), but enhance it in others (catfish and Arctic charr, Jorgensen *et al.* 1993). Halibut suffer less injury at high densities (Greaves 2002) but show more abnormal swimming (Kristiansen & Juell 2002). The relationship between welfare and density may be non-linear; low densities may harm rainbow trout, in salmon negative effects start to appear at a critical density and density interacts with other factors such as disturbance or water quality (Ewing & Ewing 1995, Bell 2002, Scott *et al.* 2001).
Enforced social contact	Aggression can cause injury in farmed fish, especially when competition for food is strong (Greaves & Tuene 2001). Subordinate fish can be prevented from feeding (Cubitt 2002), may grow poorly and are more vulnerable to disease (reviewed by Wedermeyer 1996).
Water quality deterioration	Many adverse effects of poor water quality have been described, with different variables interacting. e.g. undisturbed salmonids use c 300 mg of oxygen per kg of fish per hour and this can double if the fish are disturbed. For such species, access to aerated water is essential for health (Wedermeyer 1996). Immunoglobulin levels fall in sea bass held at low oxygen levels (Scapigliati *et al.*1999). Heavy metals cause extensive gill damage in acidic water but are non-toxic in hard, alkaline water. (see Wedermeyer 1996)
Altered light regimes	Atlantic salmon avoid bright surface lights, except when feeding (Fernoe *et al.*1995). Continuous light increases growth in several species (e.g. cod: Puvanendran & Brown 2002).
Food deprivation	Dorsal fin erosion increases during fasting in steelhead trout (Winfree *et al.*1998). Plasma glucose increase in Atlantic salmon after 7 days without food, but other welfare indices are unaffected (Bell 2002). Atlantic salmon deprived of food for longer periods (up to 86 days) lose weight and condition, stabilising after 30 days (Einen *et al.* 1998). Farmed Atlantic salmon swim slower and fight less during feeding bouts when fed on demand (Andrews *et al.* 2002).
Disease treatment	Therapeutic treatments themselves may be stressful to fish (e.g. Griffin *et al.* 1999, 2002, Thorburn *et al.* 2001, Yildiz & Pulatsu, 1999)
Unavoidable contact with predators	Brief exposure to a predator causes increased cortisol levels and respiration rate and suppressed feeding (e.g. Metcalfe *et al.* 1987). Mortality and injury due to attacks by birds and seals can be high among farmed fish (e.g. Carss 1993).
Slaughter	All slaughter methods are stressful, but some are lees so than others (Robb *et al.*2000). Small, warm water fish such as sea bass killed by chilling in ice water had lower plasma glucose and lactate levels and showed less marked behavioural responses than those killed by other methods, in particular asphyxia (Poli *et al.* 2002, Skjervold *et al.* 2001). Electostunning may be less harmful for larger fish such as trout.

TABLE 3 continued

ANGLING	SOME DEMONSTRATED EFFECTS ON WELFARE
Capture – hooking	Injury and mortality following hooking is common, primarily in deep-hooked fish (Dubois *et al.* 1994; Hulbert & Engstrom-Heg 1980, Muonehke & Childress 1994).
Capture – playing / landing	Capture of fish by rod and line elicits a stress response of short duration (Gustaveson *et al.* 1991, Pankhurst & Dedual 1994, Pottinger 1998). Estradiol levels are suppressed in rainbow trout within 24h of capture by rod and line (Pankhurst & Dedual 1994).
Capture – handling	Exposure of exercised fish to air can have severe metabolic effects (lactate increase and altered acid-base balance), especially in larger fish (Ferguson *et al.* 1993). Capture and handling suppress reproductive function in brown trout (Melotti *et al.* 1992).
Retention / constraint / release	Retention of fish post-capture in either keepnets or stringers induces physiological stress responses, but recovery following release can be rapid (Pottinger 1998, Sobchuk & Dawson 1988). Hooking and handling for release can increase scale damage by 16% (Broadhurst & Barker 2000), possibly making released fish liable to infection. Abnormal behaviour can occur following release after a stressful event (Mesa & Schreck 1989, Olla & Davis 1989).



KEEPING ORNAMENTAL FISH	SOME DEMONSTRATED EFFECTS ON WELFARE
Capture. Exposure to Poisons	Marine tropical fish captured by sodium cyanide suffer very high mortality for several weeks after capture (Hignette 1984). Clove oil is a better alternative (Erdmann 2002).
Transportation	Estimates for mortality during capture of ornamental fish from South America range from 5 to 10% but may be as high as 30%. A further 5 to 10% mortality is estimated to occur during transportation and at the holding facilities (Ferraz de Olivera 1995). During the acclimation period following importation mortalities can be up to 30% (FitzGibbon 1993). However, in all these aspects of the ornamental fish trade there is a great deal of variability. The Ornamental Fish Trade Association has regulations to improve all aspects of capture and transport of fish (www.aquariumcouncil.org).
After purchase, constraint in a confined space	See above, under aquaculture.
Handling	See above, under aquaculture.
Inappropriate densities/species combinations	Lack of appropriate social environment (wrong species or inappropriate numbers) is an important cause of poor health in ornamental fish (Etscheidt 1995).
Poor water quality	81% of ornamental fish are held outside the optimal pH range, 36% at inappropriate temperatures (Etscheidt & Manz 1992). Poor water quality is the commonest cause of mortality in ornamental fish (Schunck 1980).
Deprivation of social contact	Angelfish transferred singly to a new tank take longer to resume feeding than those transferred in groups of 3 or 5 (Gomez-Laplaza & Morgan 1993).
Inappropriate feeding regimes	Inappropriate range and types of food can cause poor health in ornamental fish (Etscheidt 1995). Inappropriate feeding is not usually a direct cause of mortality in ornamental fish, but can be a contributory factor (Schunck 1980).
Unavoidable contact with a predator	In 19% ornamental tanks prey were housed in small tanks in direct contact with predators (Escheidt & Manz 1992, Foggitt 1997). See above under aquaculture.
Disease treatment	See above under aquaculture.

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AFS Policy Statement #30: *Responsible Use of Fish and Other Aquatic Organisms*

(Full Text)

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Issue Definition

The objectives of the American Fisheries Society (AFS), as cited in Article I, Part 2, of the Constitution, are to "promote the conservation, development, and wise use of fisheries," and to "promote and evaluate the development and advancement of all branches of fisheries science and practice." As inferred from its mission and objectives, the fundamental position of the AFS is that responsible human use of, and interaction with, fish and other aquatic organisms through management, research, and education is appropriate, desirable, and ethical.

Multiple interests in aquatic organisms and their use, however, may result in conflicting views about appropriate uses of specific fisheries. These interests may include those of commercial fishers, subsistence fishers, recreational anglers, trophy anglers, ceremonial users of fish and other aquatic organisms, naturalists, animal liberationists, and environmental activists as well as those who affect aquatic systems through industry and other forms of economic development. This wide range of perspectives leads to fundamental conflicts regarding the interaction of humans with fish and other aquatic organisms.

Conflicts over human interactions with aquatic organisms have the potential to hinder management efforts aimed at providing human benefits from fisheries and ensuring long-term ecological sustainability. These conflicts may lead to threatening confrontations. Congress passed the Animal Enterprise Protection Act of 1992 (P.L. 102-346) to protect persons engaged in almost any business related to animal industries from interference and damage from animal rights protests. Since then, participants in the animal rights movement in numerous states have pushed ballot initiatives, referenda, and statutes that pose the potential to significantly restrict all forms of fishing, conservation and fisheries management, and the use of fish and other aquatic animals in scientific research and education (Reiger 1997). Various anti-fishing protests have occurred in several countries around the world, consequently ending catch-and-release fishing in Germany and use of bait fish in Norway and the Netherlands (Spitler 1998). A review of the print media suggests that these protests have not yet posed a threat to fisheries management in North America that they have in Europe (Henson 1997; Spitler 1998). Here, we address this debate and concern (e.g.,

Wywialowski and Reese 1991; Gasson and Kruckenburg 1993; Quinn 1995; Ott 1995) about the human use of fish and other aquatic organisms.

The AFS is committed to all branches of fisheries science and communication among fisheries professionals and between fisheries professionals and the public. The AFS recognizes the diversity of perspectives within our own membership and in the world but is committed to (a) making sustainability of the aquatic resources the common goal that brings together this diverse membership and (b) supporting diverse human uses of aquatic organisms that are consistent with this goal. The purpose of this position statement is to affirm explicitly that the AFS supports the broad range of consumptive and nonconsumptive human interactions with aquatic organisms in a manner that ensures long-term ecological sustainability.

Background

Both the mission statement and the constitution of the AFS list the following objectives:

1. to promote the conservation, development, and wise use of fisheries;
2. to promote and evaluate the development and advancement of all branches of fisheries science and practice;
3. to gather and disseminate to Society members and the general public scientific, technical, and other information about fisheries science and practice through publications, meetings, and other forms of communication.

In this position statement, we review conventional and nonconventional uses of fish and other aquatic organisms, and importance of those uses; we summarize actions taken by the AFS and others to promote animal welfare in laboratory and field work; we examine the importance of sustainability and the need for management to maintain sustainable fisheries and ecosystems; we provide guidance on addressing conflict and finding collaborative ways to consider the diversity of stakeholder interests; we describe actions that should be taken by the AFS; and we provide eight policy guidelines on the use of fish and other aquatic organisms.

Conventional Uses of Fish and Other Aquatic Organisms

Human use of fish, mollusks, and crustaceans for food is pervasive worldwide. According to the Food and Agriculture Organization of the United Nations (FAO 1994), 234 countries or geographical locales, occurring on all major continents, harvest fish and other aquatic organisms. A review of fisheries statistics of these countries indicates a broad spectrum of human societies, peoples, and ethnic groups who participate in fisheries-related activities. Fish harvested for food may be for commercial, subsistence, or ceremonial uses. The world harvest is comprised of 82.5% marine and 17.5% freshwater fishes. From 1950 to 1994, world fish harvest increased from 20 million metric tons (mmt) to 110 mmt. For all fish and fishery products during the 1983 to 1993 period, 71%–72.4% was for human consumption, and the remainder was for animal feed (FAO 1993). Numbers of aquatic species harvested ranged from 54 to 234, and import/export values of fishery products ranged from $2 billion to $14 billion for the top 6 ranked countries (FAO 1994).

Recreational fisheries also play a prominent role in human society in industrialized countries. In the United States in 1991, approximately 45.1 million people (nearly one-fifth of all residents) participated in fishing recreation and spent 511 million days, 454 million trips, and 24 billion dollars (USFWS and Bureau of the Census 1993). These anglers consisted of 72% males and 28% females from all age categories, all income brackets, and all educational backgrounds. Twelve percent to 38 percent of the population in each of the 50 United States participated in fishing recreation. In contrast to commercial fishing, the majority of angler participation, time, effort, and dollars spent in

and on recreational fishing occurred in freshwater rather than saltwater. Numerous studies have documented the central importance of aesthetic, psychological, spiritual, educational, and other non-catch values to recreational anglers (Moeller and Engelken 1972; Knopf et al. 1973; Driver and Knopf 1976; Martin 1976; Hendee and Bryan 1978; Harris and Bergersen 1985; Fedler and Ditton 1994; Malvestuto and Hudgins 1996).

Agencies that manage recreational fisheries often use catch-and-release regulations as a management tool to maintain fish population abundance, fish size, and fishing quality in important sport fisheries. Catch-and-release fishing as a conservation and management tool has been shown to be an effective approach for sustaining native cutthroat trout (*Oncorhynchus clarki* spp.), rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), and black bass (*Micropterus* spp.) fisheries in Yellowstone Park, Colorado, Idaho, California, British Columbia, Oregon, Missouri, and Wisconsin (Barnhart and Roelofs 1977; Hunt 1981; Anderson and Nehring 1984; Espegren et al. 1990). In these cases catch-and-release regulations were implemented to conserve fish populations and fisheries by eliminating the harvest component of fishing. The results of this regulation, in terms of protecting fish population abundance and maintaining good-quality fishing through high catch rates and large fish size, have been so successful that the regulation has been incorporated widely in fisheries management. The application of catch-and-release regulations with respect to broader ecological management objectives and benefit to fisheries resources is consistent with the AFS position statement on special fishing regulations as a management tool (AFS 1995).

Cultural, Educational, Spiritual, and Other Nonconventional Uses of Aquatic Organisms

While primary objectives of fisheries management are considered to be catching and consuming fish, other values associated with the fishing experience have long been recognized. The justification for managing aquatic resources and fisheries is as much to provide fishing opportunities, and thus provide an array of nonharvest values/products, as it is to provide the more recognizable catch-related and other resource management products (Driver and Knopf 1976). Martin (1976) recognized the role of aesthetics as a contributing incentive to participate in fishing. Moeller and Engelken (1972), Driver and Knopf (1976), and Harris and Bergersen (1985) found non-catch, aesthetic, and psychological values often ranked above catch-related indices. In their study of understanding angler motivations, Fedler and Ditton (1994) suggested that the value of fishing for pleasure was prevalent in literature records from 300 B.C. to 1496 to 1953. These motivations or satisfactions, which are the basis for sociocultural values, vary among individuals and include a variety of factors (Knopf et al. 1973; Martin 1976; Hendee and Bryan 1978; Fedler and Ditton 1994; Malvestuto and Hudgins 1996). Temporary satisfactions and pleasures derived from fishing lead to enduring benefits that are personal, physical, psychological, and emotional (Hendee and Bryan 1978). From their reviews of human dimension factors in fisheries, Fedler and Ditton (1994) suggested that a fuller recognition of the social, psychological, and physical benefits associated with sportfishing may be critical to the continued protection of fish stocks.

Social benefits also accrue from the educational use of data and information from management and research activities. Scientific information is translated to promote the intrinsic value of aquatic systems to users and the general public (Malvestuto and Hudgins 1996). Public education can enhance wise resource use and generate public appreciation of aquatic systems (Angermeier and Williams 1994). Assessing the economic value of nongame fishes and nonconsumptive uses of fishes by the public is difficult, but this assessment is recognized as an important component in the valuation of fishery resources (Gordon et al. 1973; Talhelm and Libby 1987). Loomis and White

(1996) demonstrated that economic values can be expressed for the conservation of nongame fish species as well as for fish species with considerable commercial or recreational value, and this value is distinct from the species' use value.

Fisheries managers and researchers have tried to evaluate and maximize both the enjoyment of the sportfishing experience and the economic value of fish, fisheries, and aquatic ecosystems. Their observations and data repeatedly confirm the wide variety of important human values associated with fishing. Catching and consuming fish are only parts of the value, especially in recreational fishing. These other values and benefits should not be disregarded when assessing the ethics of human use of fishes and other aquatic organisms.

AFS Concerns for Animal Welfare

Concerns regarding the welfare and use of animals in medical laboratory research have resulted in laws, government policies, procedures, and protocols that require humane treatment of animals for all uses. Research projects, particularly those conducted in laboratories, are reviewed to ensure that projects using animals are necessary and conducted as humanely as possible. Examples of animal welfare protection laws, policies, and institutions include the Animal Welfare Act (7 U.S.C. 2131 et seq.), *Guide for the Care and Use of Laboratory Animals* by the National Institutes of Health (1985), and federally mandated "institutional animal care and use committees" (IACUC), which are in place at federally funded institutions to ensure that humane care is given to research animals.

To build on and extend these ethical guidelines to the field, thus promoting the conduct of all fisheries work in a humane manner that eliminates cruelty and minimizes suffering, the AFS—in cooperation with the American Society of Ichthyologists and Herpetologists, and the American Institute of Fishery Research Biologists—developed and subsequently published "Guidelines for Use of Fishes in Field Research" (Nickum 1988). This document responded to the 1985 amendment to the Animal Welfare Act that extended principles of humane laboratory animal care to field research activities. The AFS encourages its members to uphold public standards of humane treatment, both in the field and the laboratory.

Conservation Management and Ecological Sustainability

Recent assessments of the status of aquatic species, both worldwide and in North America, concluded that significant percentages of fish, amphibian, freshwater mussels, and crayfish are declining toward an imperiled status (Williams et al. 1989; Williams et al. 1993; Moyle and Yoshiyama 1994; Warren and Burr 1994; IUCN 1996; Taylor et al. 1996). Eipper (1995) concluded that the global human population of 5.6 billion people was overtaking fish production since declines were evident in 9 of 17 major ocean fisheries. From a habitat perspective, significant modifications to aquatic habitats have been documented by Benke (1990) for streams and rivers, by Meador (1996) for water storage and transfer projects, and by Armour et al. (1994) for western riparian and stream ecosystems. By any assessment during the last decade, there can be little doubt that humans have had, and are having, a significant negative impact on the diversity of aquatic organisms and the quality of aquatic habitats. Conservation management and research on fish and other aquatic organisms are beneficial and in some cases necessary to survival of the growing number of imperiled aquatic species (Williams et al. 1989; Angermeier and Williams 1994).

There is a growing movement in public land and water resource management away from the traditional paradigm based on maximum sustained yield toward an emerging paradigm based on

ecosystem management (Cortner and Moote 1994; Gresswell and Liss 1995; Kellert 1996; Malvestuto and Hudgins 1996). Within the fisheries field, management and research continue to move from a single-species, single-product-oriented approach, to a multi-species, ecosystem-integrity-oriented approach based on principles of ecosystem-based management (MacCall 1986; Dombeck 1996; Malvestuto and Hudgins 1996; Schramm and Hubert 1996; Starnes et al. 1996; Wiley 1996; Wiley and Gregory 1996). Ecosystem-based management recognizes the intrinsic value of ecosystem health and integrity, and recognizes human society as part of the system. Ecosystem management includes consideration of human societies, technology, economies, needs, health values, and sociocultural values as well as environmental health, integrity, and biodiversity (Malvestuto and Hudgins 1996; Schramm and Hubert 1996).

Addressing Diversity and Conflict

The changing themes in fisheries management and science are reflected in statements from recent Fisheries documents. For example, the definition of a fishery has expanded from exclusively consumptive uses to encompass a diversity of nonconsumptive uses (Gresswell and Liss 1995; AFS 1996; Starnes et al. 1996). Not surprisingly, recent AFS position statements recognize the importance of consumptive and nonconsumptive uses, and emphasize the need to protect aquatic ecosystems with all their species and ecological processes (Armour et al. 1994; Starnes et al. 1996; Winter and Hughes 1997).

The trend toward multiple-species management was accompanied by societal recognition of the intrinsic value of fish in the 1950s and 1960s (Royce 1988; Wiley 1996). Environmental political action and environmental legislation, coupled with the decline of the expansion era of world fisheries during the 1970s, led to the more recent increase of public involvement in resource policy and decision making (Royce 1988; Alverson 1995). Changes in the fisheries field continue today as demands on the resource increase (Frye and Gottschalk 1995) and diversify (Redmond 1994; Gresswell and Liss 1995; Kellert 1995, 1996). In light of this diversification, it is difficult to predict future demands on fisheries resources. However, we have found strong and clear guidance for dealing with confusing changes in fisheries in our own AFS literature.

The fisheries management charge states that management agencies are stewards of the entire fishery resource, including, but not limited to, sport or commercial fisheries. Ecosystem-based management requires sustaining nontarget fishes and aquatic species that are important components of aquatic ecosystems, in addition to sustaining species caught by people in consumptive fisheries (Wiley 1996). As part of fisheries management, we should ensure a diversity of consumptive and nonconsumptive opportunities for a wide range of public interests where consumptive uses might involve commercial, recreational, ceremonial, or subsistence fishing (Fraley 1996; Starnes et al. 1996).

Fisheries management policies ultimately are driven by a variety of factors, including scientific research and political, economic, aesthetic, and social values. Societal values are the raw materials from which fisheries management policies are forged (Wiley 1996). As primary stakeholders in the management of natural resources held in public trust such as fisheries and aquatic systems, the public directs their use and management by fishery professionals according to the public's needs for nutrition, recreation, employment, income, and a healthy human environment (Dochoda and Fetterolf 1987). Thus, "the resource professional's first priority should be to work with user and interest groups, academia, local communities, and others to develop shared goals for healthy ecosystems" (Dombeck 1996). Fisheries professionals represent the fishes and the people who use and appreciate them; they cannot make the choices among various stakeholders of watershed resources. "Only society can make the choices requiring such Solomon-like wisdom" (Wiley 1996).

As a Society, we recognize that diversity in decision-making processes is not an obstacle, but a strength (Coutant 1996; Keefe and Young-Dubovsky 1996). To draw on this strength, we must be able to understand and appreciate diverse views (Daigle et al. 1996). With respect to human interactions with animals, there is a continuum of interests and philosophies among different fisheries resource stakeholders, including fisheries professionals. The AFS membership itself is diverse, spanning 75 countries, which suggests a diversity of philosophies.

Our Society encourages diversity (Coutant 1996; Keefe and Young-Dubovsky 1996); therefore, we must expect conflict and be prepared to manage it responsibly. To address conflicting demands, conflict management experts (such as those at the Center for Conflict and Change, Humphrey Institute, University of Minnesota) suggest applying one of various conflict management methods. Conflict management methods are significantly less expensive and time-consuming than litigation and various forms of political action (e.g., legislation, ballot measures). By promoting conflict management approaches, AFS would help reduce exposure of fisheries professionals to more costly alternatives. In one example of collaborative decision making, a citizen task force was formed to determine goals for deer herd size and management strategies within a metropolitan area (Curtis et al. 1993). Conflict management methods that acknowledge diverse interests make all stakeholders feel their concerns have been considered, even if the outcome is not ideal for any single party (Richards and Krannich 1991; Wywialowski and Reese 1991). Also, if decisions can be made based on the input of many different perspectives rather than one or two, they are likely to be more sound, imaginative, and inspired (Covey 1989).

Considering the changing context of fisheries resource management and the increasing diversity of AFS and its goals, we need to expect and accept conflicting interests among fisheries resource stakeholders. AFS First Vice-president Christine Moffitt (1996) cautioned, "We must provide leadership in bringing together the profession to help resolve disputes; the Society cannot become the arm of any one interest group and survive." Also, in a recent survey of managers and supervisors in all state fish and wildlife agencies nationwide, 82% reported they could use help solving management problems. Specifically, skills in conflict management were listed second in order of priority (Angus 1996).

Needed Actions

In developing and implementing actions to address social conflicts over fishing and other human interactions with aquatic organisms, AFS needs to simultaneously (a) support fishing and other uses of aquatic organisms within the bounds of ecosystem-based management and (b) uphold freedom of expression within legal boundaries, sincerely respecting diversity in peoples' perspectives. Thus, the policies described below encourage AFS members to "review their roles in the democratic social contract, both as public servants and as citizen advocates of their beliefs, so...they hold their own conduct and that of all participants in the debate to the highest standards of integrity" (Henson 1997).

The AFS should continue to raise member awareness of the various approaches to conflict management such as facilitation, mediation, or arbitration. Supporting the use of these approaches among members will help develop inclusive and just processes in which to address conflict about issues raised in this policy statement as well as future areas of conflict within AFS and between AFS and broader society. Because members may have limited time and resources available to deal with conflict, AFS should develop a resource base of experts and educational materials to provide the necessary support for its members, including continuing education workshops on conflict

management skill building. The AFS also should exchange resources with other organizations facing similar challenges.

Policy

It is the position of the American Fisheries Society that

1. Diverse forms of utilization of fish and other aquatic organisms are prominent around the world and will continue to be important for sustaining human societies. Thus, AFS supports and promotes fisheries management policies and practices that provide opportunities to consume fish and other aquatic organisms in a manner that ensures long-term ecological sustainability.
2. The consumptive and nonconsumptive uses of fish and other aquatic organisms by humans contribute to the social, cultural, economic, and spiritual well-being of many societies. Through traditional uses, fish and other aquatic organisms are culturally significant to those societies. For these reasons, fish and other aquatic organisms will continue to be important in sustaining human societies.
3. It is appropriate and often necessary for humans to manage fish and other aquatic organisms to sustain and protect their populations, communities, and habitats, and to maintain the integrity of evolutionary and ecological processes that create and support the diversity of aquatic organisms. As the species with the greatest capacity to affect aquatic environments due to the pervasive effects of human population growth, technology, and consumption, humans have an obligation to maintain and restore aquatic ecosystems and their biotic components.
4. It is appropriate for humans to use fish and other aquatic organisms in a responsible manner for scientific, commercial, educational, cultural, and recreational purposes to promote the quality of human life, promote the quality of aquatic ecosystems, and enhance the capacity of human societies to value and conserve these ecosystems.
5. Managers of recreational and commercial fisheries should use practices that do not threaten the viability of populations of native species of aquatic and terrestrial organisms, their habitats, and their ecosystems. Management decisions should be evaluated and justified a priori in a manner that ensures long-term ecological sustainability.
6. Human interaction with aquatic organisms is governed largely by cultural mores, and different human cultures place different values on fish and other aquatic organisms. It is each individual's right to choose (within the bounds imposed by his or her society) whether or not to engage in consumptive or nonconsumptive use of aquatic organisms. It is the role and responsibility of fisheries professionals to inform societies about the implications and consequences of use of, or actions affecting, fish and other aquatic organisms.
7. All fisheries-related activities involving use of fish and other aquatic organisms, including resource management, research, administration, education, and law enforcement, should be developed within and justified by conservation principles and philosophies. Any use should be conducted in accordance with the best scientific and professional information available and consistent with humane practices, including those outlined in "Guidelines for Use of Fishes in Field Research" (Nickum 1988) and *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health 1985).
8. Fishery professionals should support and promote conflict management methods to address disagreements over fishing and other human interactions with aquatic organisms. These methods should include facilitation, mediation, arbitration, negotiation, or collaborative decision making. The success of various approaches should be evaluated so fisheries professionals can recommend ways to resolve future conflicts and determine how approaches could be altered to increase their effectiveness.

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AFS Policy Statement #22:
Commercial Aquaculture
(Abbreviated)

Robinette, H Randall (Chair); Hynes, Julian; Parker, Nick C; Putz, Robert; Stevens, Robert E; and Stickney, Robert R (1990) ABBREVIATED, AFS Policy Statement #22: Commercial Aquaculture. Approved Aug 1990, Pittsburgh, PA Published Jan-Feb 1991, *Fisheries* 15(2):12
http://www.fisheries.org/Public_Affairs/Policy_Statements/ps_22a.shtml

Recent successful commercial culture of channel catfish, salmonids, and crayfish has led researchers and entrepreneurs to investigate and invest in a wide array of potential finfish, crustacean, and molluscan candidates for commercial rearing. Commercial production is essential in meeting increasing demands for food fish and for sport fishing, the provision of aquarium fishes and bait fish, and the local production of fish to replace imports. Such rapid expansion of the industry, however, has raised many AFS concerns. Use conflicts in both inland and coastal waters can be expected to intensify as aquaculturists, recreationalists of all types, developers, environmentalists, and commercial fishers contend for use of the same bodies of water.

Disease problems, genetic pollution, escape of exotic and introduced species, and eutrophication are areas of greatest concern. There is the possibility of amplifying pathogenic organisms in an intensive culture system which might be released with or without fish into wild populations. All states and provinces should have fish health programs, but because of the diverse nature of these programs, only the federal government may be able to consistently apply equal standards throughout the country.

Biotechnologies are now providing mechanisms to genetically manipulate organisms to promote economic advantages through increased growth rates, sex reversal, etc. However, the effects of the escape or release of these genetically altered organisms into the natural environment are not known. It is imperative that aquaculturists understand the need for and that governmental agencies enforce regulations to safeguard wild populations from escaped aquaculture species, whether genetically altered or exotic.

Successful commercial aquaculture usually implies a highly intensive management system that often results in nutrient-rich effluents. Since many factors (ratio of volume of receiving water to effluent, frequency of discharge, nutrient load, geographic location, etc.) are involved in each aquaculture operation, acceptable standards should be set and enforced by regulatory agencies to avoid eutrophication of receiving waters.

In supporting the orderly development of aquaculture, and to protect the integrity of native aquatic communities, the AFS advocates the following principles:

1. Federal, state, and provincial agencies should cooperate to ensure the health of aquatic organisms, control the transfer and introduction of aquatic organisms, and inspect processing plants and fish and fish products to safeguard human health.
2. Use of organisms native to each facility's region is strongly encouraged.
3. When commercially cultured fish are considered for stocking, every consideration should be given to protecting the genetic integrity of native fishes.
4. Aquaculture facilities should meet prevailing environmental standards.

Aquaculture is a form of agriculture. The principle responsibility for development of aquaculture is in the private sector. Government should support these initiatives directly through research and development, fish inspection, and fish health certification, and indirectly by reducing unnecessary regulatory constraints, mediating in resource user conflicts, and coordinating the involvement of a diversity of government departments.

The AFS policy regarding commercial aquaculture advocates:

1. Interagency cooperation and coordination of state, provincial, and federal fisheries and aquaculture programs.
2. Passage of aquaculture legislation which creates coherent federal, state, and provincial aquaculture programs and appropriation of funds to implement the legislation.
3. Passage or amendment of food safety legislation to establish fish inspection programs to ensure the safety and quality of aquacultural products.
4. Continued development of regional and provincial aquaculture research and extension centers.
5. Development of federal, state, and provincial centers to compile and disseminate aquaculture information.
6. Improved joint programs of the federal, provincial, state, and private sectors to facilitate the use of commercially grown fish--with proper safeguards--for fisheries enhancement and mitigation, and the provision of recreational fisheries.
7. Secondary school curricula and college and university programs to train students for future employment in all aspects of aquaculture.

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Animal Welfare Issues

Responsible Care and Health Maintenance of Fish in Commercial Aquaculture

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Introduction

Production agriculture has enabled the United States to become the best fed society in the world and is vital in domestic and international trade. An important segment of production agriculture, livestock production, has successfully provided consumers with a low-cost, wholesome protein source. Consumers have dictated low prices and high quality. Consequently, to maintain competitiveness, producers have set management objectives that maximize production while minimizing costs. In addition to public concerns about cost and wholesomeness of animal products, there is growing concern for the well-being of the animals while in the care of the producers. Most animal farming systems are designed to maximize production; however, proper care and good husbandry practices are linked not only with high productivity, but also with animal health and well-being. Producers must make a conscious effort to ensure the well-being of animals in their care. The argument is often made that attention to animal welfare can adversely affect profitability. In most systems, however, improved health and well-being translate into better animal performance. Both animal welfare and environmental quality protection are the responsibility of producers, and appropriate management inputs should be factored into production costs. If production costs increase significantly, the consumer will either have to pay higher prices or face limited supplies caused by producers being forced out of business.

Aquaculture, the raising of animals or plants in an aquatic environment, has received considerable attention during the past two decades as an alternative farming practice. Aquaculture has enabled society to enjoy fish for food, pets, and recreation and contributes to the preservation of certain threatened aquatic species. Concurrent with aquaculture development, concern for the welfare of the animals grown in aquatic systems has become an increasingly important issue with certain segments of society. The goals of the aquaculturist and concerns over animal welfare are not necessarily at odds. With careful planning and proper management, fish can be cultured to meet production and profit goals while maintaining aquatic animal health and well-being.

Both the general public and the producers must understand the needs and health status of the animals being produced. However, concern should be based on scientific facts about the animals' well-being and not solely on perceptions. It is generally easier to identify with the welfare needs of animals that are more closely related to humans, such as primates and other mammals. Understanding of the well-being and care of lower vertebrates, such as fish, is usually less.

Assessment of animal well-being should be based on subtle behavioral and physiological changes as well as established environmental limits.

The number of aquatic species is vast and their needs vary greatly; here we mainly discuss finfish. New species of fish from the approximately 20,000 species worldwide are being evaluated and adopted as candidates for aquaculture. The optimum health requirements for major farm-raised species are known. However, requirements for other species are being determined by ongoing research that aims at defining the unique limits of each. Consequently, the amount of information available concerning health requirements varies considerably depending on the species. An understanding of the health requirements for a species increases with the length of time it is commercially cultured and its economic importance. We know much more about how to evaluate the well-being of traditionally grown species, such as channel catfish, goldfish, fathead minnows, golden shiners, and rainbow trout than we do about newer aquaculture species.

This article is intended to provide aquaculture producers and the general public with scientifically based information on which to base procedures for the care and husbandry of aquatic animals raised in commercial production systems.

Finfish Aquaculture Classifications

Finfish aquaculture is commonly classified according to (1) consumer use of the farm-raised product or (2) the environmental requirements of the fish being produced.

Consumer use

- The largest group based on consumer use is food fish, with the goal being a wholesome food product for human consumption.
- Another category is baitfish, with the goal of producing a fish that is healthy when it reaches the user (sports fisherman).
- Ornamental fish for pet markets are also produced on aquaculture farms. Appearance and health of these fish are very important.
- Fish are also grown for restoration and mitigation of wild populations. The goal is to replenish fish stocks for recreational fisheries or to supplement stocks of threatened or endangered species. Maintaining a diverse gene pool is important to help ensure the long-term survival of the stocks when they are released into the wild.

Environmental requirements

Aquaculture classifications based on the environmental requirements of the fish being cultured commonly use the criteria of water temperature and salinity. Each classification has an optimal range of environmental conditions where a fish species thrives and a larger range where they survive.

- Categories based on temperature tolerance include coldwater, coolwater, warmwater, and tropical fish species.
- Salinity categories consider tolerance and restrictions based on the ionic strength of the water. These categories include saltwater, freshwater, and brackishwater fish species. Some fishes, such as anadromous species (having a portion of their life cycle in both fresh and salt water), are comfortable in a wide range of salinities and may appear in more than one category.

While these classifications are somewhat arbitrary, they are helpful when discussing basic environmental requirements and the well-being of fish grown in different aquaculture settings.

Production Systems

Regardless of the type of aquaculture based on the previous classifications, fish can be cultured in many different systems. Each type of system can have different effects on cultured animals. To address the management inputs required to maintain the health and well-being of the fish within a system, it is important to understand the type of system in which the fish are grown. Each of these systems has specific sets of conditions that can be controlled by the producer, resulting in a graded level of management responsibility.

Pond Culture

Pond systems may be classified by the level of intensification or the degree of management necessary to produce the quantity and quality of fish desired. The least intensive system offers the producer few control options, and management requirements are low. Management inputs include stocking and harvesting control to establish a balanced relationship between predator and prey species (for example, a bass and bluegill sunfish pond). The major management strategy is to control the species ratios of the original stocking and to control subsequent harvests. The productivity of fish in the culture system depends on the natural fertility of the pond.

The next level of intensification involves use of inorganic fertilizer, consisting of nitrogen, phosphorus, and potassium (NPK). Management efforts are similar to those described previously, but now the fertility and production of the pond is enhanced. The fertilizer increases production of plants (primary productivity) and of the small aquatic animal life that feeds on these plants. This increased food supply results in as much as a fivefold increase in fish production.

The productive capacity of the system can be further increased through supplemental feeding. By providing commercial feed, the number and weight of fish per unit volume of water can be greatly increased. The factor limiting production is usually dissolved oxygen. Oxygen supply usually limits the total weight of fish to about 1,500 pounds per acre per year. While the weight limit varies by species, the likelihood of oxygen depletion increases as the total weight of fish increases. It is common at this level of intensification and management to grow a single species of fish. Stocking, reproduction, and feeding rates are managed to ensure that overpopulation or excessive fish weight does not create high-risk conditions.

Management at the next intensification level includes greater control of dissolved oxygen, with the objective of obtaining even higher production rates. This type of system may accommodate 5,000 to 10,000 pounds of fish per acre, depending on the species grown and the availability of water quality management equipment. Greater inputs of feed cause increased production of waste products by the fish. If the dissolved oxygen is managed effectively, nitrogenous waste products produced by the fish usually become the next production- limiting factor. If stocking and feeding rates are not carefully controlled, the concentration of un-ionized ammonia and nitrites can increase to undesirable or dangerous levels. Nitrogenous waste becomes a limiting factor because of the limited capacity of the pond biota (primarily algae and bacteria) to convert the waste products into less harmful byproducts. The amount of nitrogenous compounds that can be effectively processed and removed on a daily basis in this type of system is about 3 pounds of nitrogen per acre per day. This translates into about 100 pounds of 32-percent protein feed per acre per day. These values can change with different climates, environmental conditions, and fish species.

All of the systems described thus far are pond culture systems that require little or no water exchange. They rely on physical, microbial, and/or photosynthetic processes to remove waste products released by the fish.

Intensive Culture

Other aquaculture systems for commercial and research use require specific management practices and typically contain aquatic stocks of high density. These systems are referred to as intensive culture systems. Fish density is usually expressed in number of fish or weight of fish per cubic foot of water and/or by the flow rate. These intensive culture systems require the highest degree of management, which is aided by system design. Intensive culture systems include net pens or cages, raceways, and recirculating systems.

Net pen or cage culture systems involve the stocking of high numbers of fish per cubic foot into enclosures placed in large bodies of water. Water quality management within the cage is one of the producer's main tasks. For good water quality, water must flow through the cage at a rate sufficient to remove the water containing the fish wastes and replace it with cleaner water containing suitable concentrations of dissolved oxygen. The next management task is to ensure that the fish are stocked at the proper density. This provides for adequate lateral swimming space and limits aggressiveness resulting from dominance behavior. Under good management, certain fish species (such as catfish) can be grown at densities as high as 10 pounds per cubic foot of enclosure. Another important management strategy is making sure that the fish are fed a complete diet — one that contains all the essential nutrients. The quality of the feed used in any intensive culture system is critical because the fish have limited access to natural food sources.

In raceway production, continuously flowing water provides fish with a high-quality environment. The water flows through the system and is discharged before the water quality degrades. Fish density of a raceway system is determined

by the flow rate and quality of the incoming water. Stocking rates are high in these systems, and the lateral swimming space requirements for each species has to be known. Water flow velocities must be maintained below a critical level to avoid excessive exercise, which can cause stress. Trout, for example, are commonly grown at densities as high as 2 to 3 pounds per cubic foot of water and catfish at 10 to 15 pounds per cubic foot of water without any adverse effects, if suitable water quality is maintained. Supplemental aeration and oxygen injection are commonly used to enhance production in raceways. Because water quality is controlled more by physical factors than biological factors, problems that result from environmental stressors are usually limited. Generally, problems in raceway systems are caused by system failure (reduction or cessation of waterflow) or the introduction of disease organisms into the facility.

Recirculating culture systems can be complex and are the most difficult aquaculture system to manage. The usual intent of this culture method is to limit new water inputs to about 5 to 10 percent replacement per day. The purpose of this control is either to control water temperature within a specific range or to limit water usage. The water that flows through the tank or trough is collected and filtered both mechanically and biologically to remove waste products before returning to the fish culture unit. Though the basic principle of this type of system is sound, backup systems are required to maintain water movement and quality within established critical limits. The most common problem with this type of system is biofilter overload or failure. Additionally, health management can be difficult because practical, legal applications of certain chemicals and drugs are constrained by unique functional features of the system. Most compounds that will control disease agents also have a detrimental effect on the bacteria that are responsible for removing or converting waste products to nontoxic forms within this system.

Fish and Their Environment

Sensory Reception and Response

The nervous system of fish is similar to that of birds, amphibians, reptiles, and mammals. Their central nervous system consists of a brain and spinal cord capable of receiving and reacting to external stimuli. The central nervous system receives information from the external environment via sensory organs and peripheral nerves. The information is processed in the brain or spinal cord, and the appropriate reactions to the stimuli are initiated. The nervous system transmits both voluntary and involuntary signals to control the action of muscles and glands. Upon stimulation, the nervous system and the endocrine glands integrate to control functions and processes such as feeding and digestion, reproduction, respiration, circulation, osmoregulation, growth, excretion, buoyancy regulation, avoidance behavior, disease resistance, and even body temperature.

While most of the endocrine and nervous system functions found in land animals are also found in fish species, there are important anatomical, physiological, and biochemical differences. A major difference between mammals and birds and most species of fish is that fish cannot control their body temperature. The temperature of a fish varies with the temperature of the water; thus also do its biochemical, physiological, and behavioral responses. While there is some variation, fish generally double their metabolic rate for each 10° C rise in temperature, within their acceptable range. This becomes important when assessing the health of fish that appear listless, a common response to low temperatures.

An understanding of how fish perceive their environment is helpful when managing their care properly. Fish are finely attuned to their environment by the senses of taste, touch, sight, smell, hearing, and additional senses unique to fish. Sense organs of fish are adapted for life in an aquatic environment and have many sensory structures and functions that differ somewhat or completely from those of land animals.

Sensory functions of fish can be grouped according to the type of physical or chemical stimuli that are detected. The detection of chemical stimuli by the senses of smell and taste may overlap because water is very different from air as a means of transport for chemical substances. Some fish have taste buds on their body that detect the taste of food at a distance. The sensitivity of detection increases as the fish gets closer to the food source. This allows them to locate food even under conditions when it cannot be seen. Fish also have sensory organs called nares, which are similar in structure and function to those in nasal passages of land animals, but it is the water rather than the air that carries the smell.

The perception of physical disturbances by fish is also different from that of higher vertebrates because the density of water is greater than that of air. Orientation and pressure recognition, along with buoyancy control, are important parts of a fish's physical sensory capacity. Hearing in fish is different from that in land animals because sound waves are received in a liquid medium, and there is no need for specialized structures to translate sound waves from the air to liquid

(the ear drum). There is also little need for the external structures that are used in land animals to concentrate sound waves from the air. A fish's lateral line system, which is a sensing system for low-frequency pressure waves, can be thought of as "touch at a distance." This system provides fish with important information about food or predators while some distance away. Additional sensory capabilities in some species can recognize and react to very low levels of electricity. The organs that receive the electrical impulses from the water help the fish to find their prey and avoid predators. This can be important when considering the possible effects of stray electrical currents that can occur in fish culture units.

Sight in fish is similar to vision in land animals. Lens shape varies considerably among species, but the eyes are functionally similar. In some fish species, the small pineal gland in the brain has sensory function in light perception. This function is thought to be responsible for circadian rhythms (biorhythms based on a 24-hour cycle) that control maturation and spawning activity. These senses may require the regulation of light intensities and daily light/darkness regimes to avoid stress in fish.

We do not know the extent to which fish perceive pain as a sensory function. We do know, however, that when fish are presented with conditions that cause pain in humans, they display an avoidance behavior. Pain, as defined in Webster's New World Dictionary, is "a sensation of hurting or strong discomfort, in some part of the body, caused by an injury, disease, or functional disorder, transmitted through the nervous system." The difficulty in assuming similarities between what fish experience and what humans experience is based on our inability to find structures in fish that are similar to those known to sense pain in humans. It is also impossible to ascribe to fish the process of conscious recognition of pain so well developed in humans. While evidence that fish have pain receptors identical to mammals is disputed (Nickum 1988), their ability to identify irritants appears to be well documented. Thus it appears important to avoid conditions that cause a violent response from fish or more subtle physiological changes that are indicative of stress.

It is impossible for humans to understand completely how fish perceive and respond to their environment. Some differences that are not part of our own experiences are how fish perceive acoustical and electrical stimuli and their ability to taste the environment with external taste buds. Possibly even more difficult to understand is how fish perceive touch. An important question to answer might be whether fish have the ability or need to discriminate between tactile stimuli that humans describe as "pleasurable" or "painful." This question is certainly important when considering the well-being of higher vertebrates that have the ability to display their pleasure or discomfort. Because we do not understand the fish's perception, a prudent policy would be to assume that conditions that cause pain in higher vertebrates should be avoided with fish whenever possible.

Environmental Stress and Disease

Fish, like other animals, have both generalized and specific responses to prolonged or repeated exposure to less than favorable environmental conditions. In a manner similar to other vertebrates, fish respond with a specific set of biochemical and physiological changes that help them survive bad conditions. Some of the changes that occur when a fish is exposed to a stressor are similar regardless of the type of stressor. The types of stressors that can occur in aquaculture are chemical, physical, or behavioral. Because the net effect of a stressor is costly to the fish's energy, stressful environmental conditions become costly to the producer and may result in lower production efficiencies and a poor survival rate.

The overall effect of a stressor on an animal depends on the nature of the stressor and the degree and duration of exposure. Three recognizable stages are common in animals forced to tolerate sub-optimal conditions: a **stage of adaptation**, a **stage of recovery**, and/or a **stage of exhaustion**. The degree and duration of the stressor generally dictates the outcome of the stress event. If the stress event is limited, fish are often able to adapt to the conditions and reestablish normal function under the new set of conditions. If the stress is removed, fish will generally go through a process of recovery, where they reestablish normal function over a period of time. If the stress is too great for the fish to compensate through adaptation, the fish will enter the stage of exhaustion and eventually die.

Even if fish recover from a stressful experience, important physiological and immunological changes can cause the animal to become more susceptible to disease organisms. The response pattern of fish is less understood than that of mammals and birds, but its key elements are similar. The basic response of fish to a stressor or adverse condition is to adopt an emergency survival status. While some of the responses that occur have obvious benefits to the fish, such as mobilization of energy reserves, other responses appear to have negative effects on long-range survival, such as decreased immune function. It appears that when fish are presented with a stressor, they sacrifice long-term survival strategies to concentrate their efforts on short-term survival.

The overall effect of a stressful environment to fish stocks is reduced performance. Reduced performance may be measured in poor survival, poor feed conversion rates, poor reproduction, and poor feeding and growth. Thus, raising fish in sub-optimal conditions is not to the advantage of the aquaculturist. Understanding the environmental requirements of the fish species and providing proper care and health maintenance to avoid stressful conditions are the keys to the success of the producer and the well-being of the fish.

Responsible Management

The basic requirements for the well-being of fish that are raised in an aquaculture facility must be provided by the producer. While fish in the wild are capable of migrating and changing behavioral patterns to meet their needs, fish in an aquaculture facility often cannot seek out optimum or more suitable conditions. To provide fish with a healthy environment, it is important to have both a properly designed facility and a management plan that addresses the needs of the fish. Fish should be provided with their basic needs: sufficient lateral swimming space; good water quality; a nutritionally complete diet; limited physical disturbance; and careful, prudent handling. The producer should also have a health management program that focuses on both infectious and noninfectious diseases. The program should be based on sound information and a thorough understanding of environmental requirements of the fish species and the culture system.

Because there is so much diversity in culture species and culture systems, a responsible management strategy has to be developed for individual aquaculture operations. For example, the management inputs necessary for a less intensive pond system raising an environmentally tolerant species such as the common carp would be low. However, raising the more environmentally sensitive rainbow trout in a recirculating system would require a very high level of management input. Proper management is a requirement for achieving high fish performance in any culture system. A well-designed and properly managed aquaculture facility can produce fish consistent with production goals while maintaining the well-being of the fish.

Stocking Rates

The number of fish stocked in the culture unit is very important to production goals and the well-being of the fish. Enough fish must be stocked to meet production goals but not so many that management cannot maintain proper health. If stocking rates exceed the carrying capacity of the system, then management to maintain acceptable conditions may be impossible. The influence of stocking rate is expressed in two ways: (1) effects fish have on the environment, and (2) effects fish have on each other. Greater fish densities will result in greater release of waste products into the culture environment. To avoid water quality problems associated with stocking rates, the capacity of the system to remove waste products should be understood by the producer. The carrying capacity of the system is limited by reliable physical and biological processes that have the capacity to remove specific amounts of waste on a reliable basis. Stocking rates should match the quantity of fish to be produced with the carrying capacity of the system. Additionally, the producer should provide the equipment necessary to maintain a healthy environment, and the management necessary to ensure production goals and the well-being of the fish stock.

Assuming that the stocking rate is within the carrying capacity of the system, the next important consideration is fish interactions. Most fish species of commercial aquaculture are characteristically tolerant of the presence of other fish of their own species. This is important in the selection of a candidate species for aquaculture. The lateral swimming space of high fish densities is most important in culture systems such as raceways, tanks, or cages. Depending on the species, limited swimming space may or may not cause stress. For example, catfish have been grown in cages in excess of 10 pounds of fish per cubic foot without a reduction in performance (Davis et. al. 1991). There is evidence that intermediate stocking rates of catfish (below 4 fish per cubic foot) results in fighting and injury. Thus, catfish raised in intensive systems should be stocked at rates that do not exceed the carrying capacity of the system and are above the threshold where fighting commonly occurs. Studies on coldwater fish (salmonids) have demonstrated that an elevated cortisol level (an indicator of stress in fish) depends more on dominance factors and interspecies fighting than on rate of stocking (Li and Brockman 1977).

A fish's natural behavior influences its density requirement. For example, adverse effects of crowding are often experienced with open-water pelagic species and predatory species, but occur infrequently with schooling or socially oriented fish. Consequently, naturally tolerant species are ones often selected for aquaculture. It is recommended that producers carefully investigate stocking rates to establish criteria that minimize aggression among cultured fish and maintain good water quality.

Water Quality

Management of good water quality is necessary to maintain good production and the well-being of farm-raised fish. Two sets of water quality conditions must be managed. The first set consists of factors that are generally provided within an optimal range for the culture species. Examples are dissolved ions (sodium, chloride, calcium, and bicarbonate), temperature, pH, and dissolved oxygen. The second set consists of water quality factors that, in excess, are potentially harmful to the fish and should be maintained below a specific threshold. This set can be divided into (1) external or introduced toxicants, such as heavy metals, pesticides, and supersaturated gases and (2) natural substances, such as ammonia, nitrites, carbon dioxide, hydrogen sulfide, and suspended solids.

To maintain the health of the fish in the culture unit, it is important to select a water source that meets the requirements of the fish. A culture unit's water supply will often limit the range of species that can be grown. Not only does the ionic content of the water determine the aquatic environment where aquaculture can occur (i.e., saltwater, brackishwater, or freshwater), it can also affect management practices. For example, in freshwater aquaculture, calcium, sodium, and chlorides are very important ions to fish physiology. If they are not present in concentrations high enough for the fish to efficiently utilize them from the water, then a fish can have osmoregulatory (salt water balance) problems. The dissolved ion complex of bicarbonate/carbonate is very important in management because its buffering capacity (total alkalinity) helps control changes in water pH. While all of the important ions can be added to aquaculture water supplies, cost and logistics of such additions make certain water sources impractical for aquaculture.

Temperature of the source water is also very important in selection of production sites. As mentioned earlier, temperature is important in classifying aquaculture systems. Species-specific temperature requirements also make certain climates and water sources preferred for optimal growth (table 1). While temperature of the water can be changed to meet the requirements of almost any fish species, the cost is often excessive. Rapid water temperature changes will also cause stress in fish. It is generally recommended to change the water temperature slowly at a rate of less than 3° C per hour. This allows the fish to adapt to a new water quality condition.

Table 1. Preferred water temperature ranges for optimal growth for various fish species of different temperature classifications.

Temperature Classification	Fish Species	Optimal Temperature Range °C
Coldwater	Rainbow trout	7-13
Coolwater	Yellow perch	24-27
Warmwater	Channel catfish	28-31
Tropical	Tilapia	27-32

The type and concentration of dissolved ions in water must be compatible with the species of fish that are grown within the system. Salinity is a measurement of the ionic concentration of water, primarily sodium and chloride. The salinity of the water greatly affects the physiology of the fish being cultured. Waters can be broadly classified into three basic categories: saltwater — >20 parts per thousand (ppt); brackishwater — 5 to 20 ppt; and freshwater — <0.5 ppt. The strategy that different fish species have developed to maintain internal salt concentrations (osmoregulation) depends on the salt concentration of their natural environment. Saltwater fish have developed mechanisms that help to remove or exclude ions from internal tissues. Freshwater fish have developed mechanisms to concentrate or retain internal ions within their bodies. In fresh water, sodium and chloride should be maintained at a level of at least 10 parts per million

(ppm) and calcium at 20 ppm for most fish species. Selection of a fish species that is compatible with the water source is necessary if fish are to be raised under healthy conditions.

The pH of the water in the culture unit should be maintained within a desired range (generally 5 to 9) for the health and well-being of the fish. The pH of the water is dependent on both the buffering capacity (usually total alkalinity) and the biological activity within the unit, including the fish. The buffering capacity of the water controls the degree of pH change in the water which is caused by photosynthesis and respiration. Photosynthesis by plants in the system removes carbon dioxide (the major source of acidity in most natural waters) from the water, causing the pH to rise. Respiration, on the other hand, adds carbon dioxide to the water, thus lowering the pH. The changes in pH that occur in the system are dynamic and can differ from hour to hour depending on conditions. As with other water quality conditions, maintenance of pH within the acceptable range must be considered during facility design and managed during production.

Possibly the most important management task of a producer is to maintain dissolved oxygen at acceptable levels (above 4-5 ppm). The level of management changes dramatically with the intensity of the culture system and is also affected by the fish species raised. There are two basic approaches to managing dissolved oxygen in aquaculture systems:

- passive management, and
- active management.

The **passive management** approach is to control stocking rates so that dissolved oxygen concentrations in the water do not reach critical levels (below 4-5 ppm). Oxygen can be managed by stocking and feeding fish at low levels, as with low intensive pond culture (feeding under 30 pounds per acre per day) or by designing a raceway system so adequate water replacement keeps dissolved oxygen at desired levels. The critical level depends on the species and their health status.

The **active management** approach is to introduce supplemental oxygen by mechanical or other means. There are many different designs and approaches, but all supply oxygen to the fish at a rate that will prevent stressful conditions. The two major strategies for supplying oxygen to the fish are, (1) aeration, where the diffusion of oxygen is mechanically enhanced, and (2) oxygenation, where pure oxygen is delivered into the water. Regardless of the method used, dissolved oxygen should be maintained at acceptable levels to ensure good production and the well-being of the fish.

Of the compounds that are directly toxic to fish, the types that come from sources outside of the system (external toxicants) are the most diverse. It is necessary to prevent the occurrence of these compounds in production systems by proper site selection, water source evaluation, selection of nontoxic materials, and avoidance of any harmful contaminants.

A second group of compounds that are toxic to fish are the compounds that are produced within the system. Some of these are released by the fish as metabolic byproducts (ammonia and carbon dioxide). Others are products of decomposition of the waste products, such as nitrites and hydrogen sulfide. A third group of compounds, produced by other organisms within the system, include bacterial and algal metabolites. Fish waste products are very soluble in water and quickly become incorporated into the water. Metabolites and their breakdown products become environmental problems for the fish if released in excess of a culture system's ability to convert them to harmless forms (table 2). When more fish are raised per unit of water, the release of metabolic wastes also increases. Fish cultured at high densities without proper waste management can cause poor water quality. This increases the risk that the water will become degraded to the point where fish will experience discomfort. The metabolic byproducts of primary concern are the nitrogenous compounds; of these, ammonia and nitrites are the most important. Proper management of waste products requires careful design of the system to ensure that the waste produced by the fish is disposed of in an efficient and environmentally sound manner. It is also important to stock fish within the waste disposal carrying capacity of the system so the system does not become overloaded. To maintain proper fish health, good water quality must be provided by source and system design and through proper management based on the needs of each species.

Table 2. Critical levels of naturally occurring waste products in fish culture.

Compound	Critical Level
Ammonia	>0.05 ppm NH_3-N
Carbon dioxide	>10 ppm
Hydrogen sulfide	>0.005 ppm H_2S-S
Nitrite	>20 percent of Cl^- concentration

Nutrition and Feeding

The complete dietary requirements for all commercial aquaculture species are not known. Generally, the longer a species has been raised in aquaculture, the more is known about its specific dietary requirements. Recommendations on the protein, energy, amino acids, essential fatty acids, vitamins, and minerals are published in the scientific literature and by the National Research Council (1983) for catfish and trout. While the feed manufacturer is usually responsible for providing feed of adequate quality, producers should know the nutritional needs of their fish. Nutritionally complete rations are required for fish reared in intensive culture conditions, while those grown in the least intensive conditions can consume more natural foods that contribute to their nutrition.

Feeding practices are also very important and can change with size and developmental stage of the fish. It is important to feed the fish on a prescribed schedule according to specific nutritional needs. The amount to be fed should be adjusted as the fish grow so they receive the proper quantity of feed daily. Additionally, temperature and water quality conditions that exist prior to and at the time of feeding can also affect feeding response. Feeding activity is a very important observation in management and is often the first indication that one or more problems exist with the fish or in the production system. Any sudden decrease in feeding activity not attributed to natural variation (such as a change in temperature) should be investigated immediately, because it is likely that management action is required.

Physical Disturbances

Because fish are so attuned to their environment, it is important that tranquility be maintained by minimizing physical disturbances. For indoor systems, this should include provisions for necessary photoperiod (daylight cycle) manipulation and no sudden changes in light intensities. Avoidance of loud or startling noises is important. Care should be taken to not disturb fish by casting shadows over them or tapping on tanks. Care should also be taken to prevent stray electrical currents in production units, especially with highly sensitive species. Restricted access should be maintained to facilities where fish are raised in tanks to prevent excessive physical disturbances. Fish can also be stressed by excessive water velocity in raceways; the critical swimming velocity should be investigated for the species being cultured in these systems. Studies with trout demonstrate that water pH of less than 5 and more than 10 has a negative effect on the maximum critical swimming speed (Ye and Randell 1990). The velocity of the water in a raceway should be set at a rate (usually expressed in body lengths per second) that will effectively remove wastes but does not over-exercise the fish. Excessive turbulence caused by water flow or aeration should also be avoided, especially when culturing very small fish.

Handling Fish

Handling and harvesting can cause some of the most stressful episodes in the life of a cultured fish. This is because during handling, fish are often restrained or confined for periods of time outside water and many times are held in suboptimal water quality conditions. It is therefore very important to handle fish as infrequently as possible and with great attention to proper handling practices. The proper salt content, temperature, and other water quality conditions should be maintained when fish are handled or transported. In some cases, approved anesthetics can be used to reduce excitement of fish during transport. This can reduce fish metabolic rates and relieve stress. The addition of salt to

transport tanks for freshwater fish can also reduce the effects of stress by improving the efficiency of salt balance mechanisms. Every effort should be made to minimize the amount of time that fish are restrained or held out of water.

Health Management

Disease management in aquaculture systems begins with creating and maintaining a good living environment for the fish. Proper design and good management are necessary to minimize health risks by reducing stress to the fish. Once the system is designed properly and the management practices are directed to reduce stress, it is important to minimize the contact of the fish with infectious disease agents. Prevention is the best approach for avoiding diseases, and management plans should include a vigorous health management program including quarantine, hygiene, health monitoring, and disinfection when appropriate. Treatments should be used only after a proper diagnosis of a treatable infectious disease has been made. Use only drugs and chemicals that are FDA-approved and proven to be safe and effective. Many disease treatments can have an adverse effect on the water quality within the system. Monitor water quality and be prepared to implement management action when necessary.

Disease Prevention

Disease prevention is an important part of any animal production system. Two aspects of prevention are especially important in a health management program.

- **Avoidance** — Do not allow fish to make contact with specific pathogens. The objective is to ensure that no obligate, contagious pathogens are introduced into the facility. Management includes control of inputs, such as water supply, equipment, personnel, fish feed, and live or dead fish.
- **Stress prevention** — Maintain the animal in a healthy and robust condition by preventing stress. Effective management of stress prevents and helps reduce the number of disease outbreaks caused by facultative pathogens, which can only become established when fish are predisposed by a stressor.

Good management practices minimize introductions of disease agents by recognizing their potential sources. The most common means of infectious disease entry is introduction of infected fish from contaminated sources. Screen new fish for important diseases that affect the species being raised. This can be accomplished in part by review of historical evidence provided by reputable suppliers and through inspection (Thoesen 1991). Quarantine the fish in an isolated portion of the facility for 4 to 6 weeks at a temperature that allows outbreaks of specific diseases. Do not share equipment with other facilities, and disinfect it between uses. Personnel should take preventive action before entering a facility or areas of a facility where they can potentially spread or carry harmful disease organisms. Buy feed from a reputable source and store it properly until used. These practices are general, but they will help reduce the potential of disease introduction into the production system or farm.

Summary

Aquaculture producers should use good management practices to ensure that the animals within the culture systems meet production goals and are cared for properly. Successful production and profitability require an understanding of the needs of the fish and the use of management practices that reduce stress. The most pressing task of new producers is to learn the specific requirements of the species selected and the limitations of their culture system and water sources. This task is easy for some commercial species and systems because of past commercial successes and available literature. For new species and new kinds of systems, however, the track record and scientific information are lacking. Prospective producers should learn as much as possible about the aquatic animal and the chosen production system. Making sure that the species selection is compatible with the culture system is the first ingredient necessary for success. The producer then must make a commitment to proper system design and management. If the information on a particular species and system is sparse, the venture will be risky to the producer as well as to the fish.

Development of procedures to ensure the well-being of farm-raised fish is a dynamic process that will require ongoing research to provide new information on how to successfully culture aquatic species with minimal stress. Stress prevention, which contributes to an aquatic animal's well-being, also improves the profitability of an aquaculture enterprise. Producers should pay close attention to ensuring, through proper management practices, maintenance of a suitable environment. Proper design and management of aquaculture systems can help ensure the well-being of the fish

and production efficiencies. There are many excellent books available on the culture of aquatic organisms, design and management of aquaculture systems, water quality management, stress in fish, and health maintenance procedures. Consult with an aquaculture specialist on how to select a species and a system that has a high probability of success.

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Fish, Amphibians, and Reptiles

Guidelines for the Care and Use of Fish in Research

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INTRODUCTION

This article focuses on the use of fish as a laboratory resource. The discussion should be useful to the principal investigator and the personnel responsible for fish husbandry, care, and management. It will also aid members of institutional animal care and use committees (IACUCs) when they evaluate the proposed use of fish species in various research projects.

During the last 10 years, the use of fish in research has been increasing (DeVita 1984; Post 1987; Powers 1989; Goodrich 1990; Evans 1993; Stoskopf 1993a). The development of fish as a food source has also grown. Aquaculture is one of the most rapidly expanding new food industries partly because fish are seen as a low-fat food source and as a replacement for meats that are fat-rich. Consequently, an increasing emphasis is placed on the amount of fish consumed, the quality of fish produced, and the efficiency of fish growth.

Research into the health and husbandry of food fishes is advancing with the development of the aquaculture industry and an increasing interest in maintaining fish as pets. At some point, the U.S. Department of Agriculture (USDA) or a similar group might inspect fish to ensure their quality as a food source. Research on fish is also expanding and intensifying as a result of a greater interest in establishing new aquaria for entertainment and educational purposes. Although aquarists are interested in a wider range of species than aquaculturalists, they are in a unique position to solve special husbandry problems with special species as well as identify valuable animal models or species with particular biological properties. The attention that fish have received from both hobbyists and the fish industry has increased awareness of fish health as a factor that impacts the environment and as a measurement of environmental health (McKone and Daniels 1991).

Fish research and more specifically research in aquaculture helps address questions regarding environmental pollution, conservation, and protection of the freshwater estuarine and marine environment. Fish are increasingly used in the laboratory as animal models in toxicology. Outside of the laboratory fish are also subjected to environmental stresses (man-made and otherwise) that can harm their health and well-being.

All of these factors should also act to stimulate field research, that is, research performed directly on fish in their natural habitat, even though the number of experimental variables is much greater. Recently, as a result of greater concerns about the humane use of higher vertebrates in research, fish have been evaluated as a replacement in toxicologic, pharmacologic, and genetic studies that might otherwise employ mice or other mammalian species. All of this will increase the use of fish in research and expand the knowledge base on the care and use of these species in the laboratory.

Comprehensive guidelines on the care and use of fish would be difficult to compile as there are so many different types of fish with a variety of husbandry requirements. Fish research in laboratory animal science explores and uses their incredible diversity (Pough 1992); the 20,000 species of fish worldwide constitute about half of all living species of vertebrates, making them the largest group of living vertebrates (Schaeffer

and others 1992). In addition to size, which varies from a few to 15 meters, fish vary significantly in their taxonomy, morphology, genetics, behavior, physiology, and ecology. Currently, the Animal Welfare Act does not cover certain animals, including cold-blooded vertebrates. However, all institutions funded by the Public Health Service (PHS) must follow the *Guide for the Care and Use of Laboratory Animals (Guide)* (NRC 1996), which covers all vertebrate species. The specific use of cold-blooded species including fish is not discussed in the *Guide,* but all institutions are expected to care for and use fish in research in a manner judged to be professionally and humanely appropriate for the particular species in question. Although fish differ from both warm-blooded and other cold-blooded species, like their endothermic counterparts they need to be maintained in a controlled environment with a limitation on stress.

SPECIES--MARINE AND FRESHWATER

Certain species of fish that are frequently used in research include rainbow trout *(Oncorhynchus mykiss)* (which range from 25-30 cm in length) and other salmonids, such as coho salmon (O. *kisutch),* sockeye salmon (O. *nerka),* Atlantic salmon *(Salmo salar)* and brook charr *(Salvelinusfontinalis)* (less than 25 cm long). Toxicity testing is frequently done with fishes such as the fathead minnow *(Pimphales promelas)* (up to 10 cm), sheepshead minnow *(Cyprinodon variegatus)* (8 cm), silversides *(Menidia beryllina* and M. *menidia)* (up to 14 cm), and the Japanese reedaka *(Oryzias latipes)* (up to 4 cm). The zebrafish *(Brachydanio rerio),* which is less than 5 cm long, is currently being used extensively in molecular and genetic studies. Other species frequently studied include catfish such as channel catfish *(Ictalurus punctatus)* (up to 1.2 m), brown bullheads *(Ictalurus nebulosus)* (up to 50 cm), sunfish *(Lepomis macrochirus)* (about 20 cm), tilapia *(Oreochromis mossambicus)* (up to 40 cm), Amazon molly *(Poecilia formosa)* (3-5 cm), and American eels *(Anguilla rostrata)* (up to 1.2 m). Ornamental species are also used such as goldfish *(Carassius auratus)* (up to 30 cm) and koi *(Cyprinus carpio)* (up to 60 cm).

The UFAW Handbook on the Care and Management of Laboratory Animals (Poole 1987) has useful chapters on freshwater fish and marine fish, which also address their potential laboratory uses.

AVAILABILITY OF FISH SPECIES

Choice of Species

When selecting fish for laboratory research, the first consideration should be choice of fish species. Depending on the type of research, the species will fall into three main types: marine, freshwater, or brackish. Making this decision will determine much of the life support structure needed, as well as begin to delimit husbandry measures.

Ease of maintenance is also a principal component of species choice. Delicate animals that require special care several times a day and those that suffer higher mortality rates may not be as desirable a choice as hardier fish that require less laboratory personnel time. Factors such as diet, temperature requirements, resistance to disease, and social compatibility all need to be considered before selecting a species. Space requirements, too, should be thought out. Some species, whether because of their size or an active or aggressive nature, need larger environments, which can take up laboratory space and require larger and more expensive life support equipment.

Sources of Fish

Once a suitable fish species has been selected, the investigator must then choose between acquiring captive-bred or wild-caught animals. Captive-bred fish are supplied mainly by hatcheries and laboratory supply houses, but some laboratories, aquariums, and hobbyists are also able to provide stock. Wild-caught fish can be bought from suppliers, or collected by oneself. Individual fish hatcheries are too numerous to list in this article, but they may be identified through each state's department of natural resources, or analogous office. For a description of how fish are collected, some of the challenges they face, and the associated medical procedures, investigators can refer to *Medical Procedures Used During the Capture and Transport of Fish* (Stetter 1992). See Table 1 for an abbreviated list of fish suppliers and other resources.

Permits and Licenses

Wild-caught fish for laboratory use, whether captured by an investigator or a collector, may require scientific collecting permits. These include the federal and state scientific collectors permits and import permits.

Obtaining a state scientific collecting permit requires that one contact the appropriate governing agency (such as the department of natural resources), and request an application. The permits are not difficult to complete, last for 1 to 2 years, and are usually approved, although collecting protected or regulated species (such as striped bass, trout, or certain shellfish) may require special justification. The species sought will need to be listed by the applicant. Special collecting techniques such as electrofishing, use of certain types of nets, or use of chemicals may also call for a separate letter of authorization. The state office will require the collector to notify it where and when the collecting will take place, as well as to submit a year-end report on collection activity.

Federal permits are not needed unless one is working outside state boundaries, in federal waters, or with federally protected species. A telephone call to the Federal Wildlife Permit Office (see Table 1) will help to determine if a permit is required, as well as through which regional office the application should be routed. Having a federal permit for an endangered species will not automatically cover state requirements, however. The appropriate state agency will be able to determine if a special state license is needed in addition to the federal one. A federal listing of endangered and threatened wildlife and plants can be obtained from the U.S. Fish and Wildlife Service. Title 50 of the U.S. Fish and Wildlife Service restricts movement of certain food or sport fishes or their products from different parts of the world. Although the legislation applies only to food and sport fishes (including live and dead fish, fish products, and fish eggs), it is wise to be aware of diseases and their potential for transmission even when importing ornamental fishes for research.

When importing fish from outside the U.S., a special Declaration for Importation or Exportation of Fish or Wildlife (USFWS Form 3-177) must be completed and filed with the local U.S. Customs agent. This may be accompanied by an inspection by a Customs agent.

Fishes that appear on the Convention on International Trade in Endangered Species (CITES) lists are strictly controlled, and without the proper authorization, importation is prohibited. Appendix I of CITES lists animals that are threatened with extinction, and their acquisition is highly discouraged except under the most exceptional circumstances. CITES permits commercial trade in species listed in Appendix II, provided that the country of origin has issued an export permit. Appendix III species may be traded; listings are made in order to gain recognition and protection for species that are in danger of becoming threatened.

SUMMARY OF APPLICABLE ANIMAL WELFARE LAWS AND GUIDELINES

Any vertebrate research performed or sponsored by the U.S. government is covered by the *U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (PHS Principles)* (PHS 1986 p 27). For guidance in following these principles, investigators are directed to the *Guide* (NRC 1996), which recommends that an "appropriate environment be provided" for nontraditional species and that "expert advice on the natural history and behavior of nontraditional species" be sought (NRC 1996, p 14 of prepublication copy). Except for a short bibliography, fish-specific information is not included in the *Guide*. Other useful guidelines include *The Care and Use of Amphibians, Reptiles and Fish in Research* by the Scientists Center for Animal Welfare (SCAW) (Schaeffer and others 1992) and *Guidelines for The Use offish in Field Research* by the American Society of Ichthyologists and Herpetologists, the American Fisheries Society, and the American Institute of Fisheries Research Biologists (ASIH 1987). Also useful is the Canadian Council on Animal Cafe's *Guide to the Care and Use of Experimental Animals* (CCAC *Guide)* (CCAC 1989). The Animal Welfare Act does not cover fish or any of the cold-blooded vertebrates.

The *PHS Principles* recommend selecting the appropriate species of the appropriate quality, as well as using the minimum numbers of animals required for valid results. Nonanimal alternatives, discomfort, distress, and pain must also be considered. Living conditions should contribute to the health and well-being of the animal, and animals should be cared for by trained and experienced personnel, inclusive of veterinary care. Investigators and personnel should be qualified, experienced, and receive training if necessary. Any exceptions to the *PHS Principles* must be reviewed by the IACUC. All of the above conditions are directly applicable to research with fish.

In addition to consulting with the attending veterinarian of the facility, it is frequently valuable for the IACUC to have access to regular ad hoc members that are full-time specialists in the maintenance of fish as research animals. Individuals with relevant backgrounds could be scientists, aquarists, curators, directors of husbandry, or veterinarians working in aquariums or aquatic research centers.

DEVELOPING PROCEDURES FOR HOUSING, HUSBANDRY, AND BREEDING

Centralized Facilities

Since there are over 20,000 species of fish that live under many differing environmental conditions, husbandry will vary depending on the natural environment for a particular species. Life-support systems must be set up and "acclimated" prior to receiving animals (Spotte 1979, 1981; Alabaster and Lloyd 1980; Hawkins and Lloyd 1981; Munro and Roberts 1989; Goodrich 1990). During construction, thought should be given to providing adequate space and water quality. If a municipal water supply is used the water must be either dechlorinated by aeration or by filtration through activated carbon or chlorine-precipitating compounds such as sodium thiosulfate. All water should be analyzed prior to use, whether municipal or well water. Investigators must be aware of the appropriate levels of pH, ammonia, nitrate, and calcium in the water medium (Tucker 1993).

Laboratory fish rearing systems include static aquaria, flow-through, closed water recirculating, ponds or lakes, and a net or cage placed in a body of water (Schreck and Moyle 1990; Moe 1992). The first two methods are commonly used in fish research laboratories. Although flow-through systems have many advantages and are recommended, they are very expensive to set up and maintain. In the closed water system, which is more practical, water management is crucial.

Construction

Careful planning is crucial in choosing materials (concrete, plastic, or fiber) for construction of tanks, tank covers (to prevent fish loss and contamination), and plumbing. The tanks must be reasonably inert to the water. Care should be taken to ensure that concrete structures are properly treated so that there is minimal leaching and salt deposition (Hawkins and Lloyd 1981). Construction materials should not contain copper, nickel, cadmium, or brass. Polyvinyl chloride pipes are commonly used, but once installed the system must be adequately flushed to eliminate acetone, methylethylketones, and tetrahydrofurans that are released following gluing (Reimschuessel and Kane 1993). This may take weeks and requires water chemical analysis to confirm that the toxins have been removed.

Water Quality

Biological filtration, mechanical filtration, chemical filtration (adsorption), and disinfection are four major processes used to maintain closed water systems (Spotte 1979). Biological filtration involves heterotrophic and autotrophic bacteria that convert nitrogenous organic compounds (fish excreta, consisting primarily of ammonia) into nitrites and further to the less toxic nitrates (Stoskopf 1993b). Investigators should monitor the nitrification process, which is affected by many factors, including temperature, pH, dissolved oxygen, salinity, surface area of the filtrant, and bactericidal and parasiticidal agents.

Mechanical filters capture and eliminate undissolved particulate matter and organic particles that would otherwise contribute to the nitrogenous waste. These filters must be properly maintained to ensure that the water flows correctly and that detritus is eliminated. Chemical filtration, including granular activated carbon, foam fractionation, and ion exchangers are used to reduce organic carbon, ammonia, nitrate, and phosphate. Disinfection methods and ozonization and UV light treatment have been used to oxidize organic matter and to kill bacteria in both flow-through and recirculation systems (Schreck and Moyle 1990).

Temperature

The health, nutrient requirements, performance, reproduction and, in extreme cases, survival of fish are all dependent on the temperature of the water. Temperature requirements vary among species as well as between estuarine and freshwater fish (Tomasso 1993). Very gradual equilibration of water temperature is crucial when transferring, shipping, breeding, and acclimating fish, as well as when adjusting water

temperature. (Extreme caution must be taken with all electrical connections. Tanks should be well insulated and grounded and appropriate ground fault circuit breakers should be used.) An optimal temperature variation is about 1 °C/hour (Tomasso 1993), which is not considered stressful. Temperature variation also affects saturation of gases including oxygen (for example, there is less dissolved oxygen at higher temperatures). Gas content is inversely proportional to the water temperature. Sudden, large increases in temperature pose a hazard to the fish, particularly in a closed container aquarium.

Illumination

Both photoperiod and light intensity are important and requirements vary among species.

Periodicity. Although most species do well with a cycle of 12 hours of light and 12 hours of darkness, 8-10 hours of light is generally adequate for most fish, while 12-14 hours is appropriate for tropical fish (Moe 1992). This dusk to dawn system can be set up with a timer that turns a low light on and off for 30 minutes preceding and following the main tank lights.

Quality of light. Fluorescent lighting is commonly used in aquaria. Generally, an intensity of 10-12,000 lx of full spectrum lighting over the water surface can be used. A color temperature of 5000-7000 K and peak wave length ranging of 475 and 650 mm at proper intensity will provide good quality lighting. The readers are directed to an excellent extensive discussion on the quality of light by Moe (1992).

Diet

Fish are one of the most efficient animals in converting food nutrients into body tissues. This efficiency is due to a number of factors--they are poikilotherms, they excrete waste efficiently, and they require little energy for support and transportation (Halver 1989). In addition to the basal metabolic rate, temperature, stress, and health status are important determinants of energy requirements. Diets should be compounded with the above factors in mind while making sure that the energy requirements include all essential nutrients.

Essential amino acids (proteins make up 60-70% of fish tissue on a dry weight basis), vitamins, and minerals must be in proper ratios to ensure a well-balanced diet. Diet preparation must be appropriately performed to achieve specific research needs, to ensure sufficient stability in water, and to minimize pollution (Hardy 1989).

pH

Levels of pH between 6.5 and 9.0 are desirable, pH has multiple effects on the levels of dissolved gases and dissolvability of metals in the water as well as on oxygen uptake by the fish. It also affects organic acids and phosphates and the ratio of non-ionized to ionized ammonia. Fish vary in their tolerance to pH at different stages of their lives. Levels of 6.5 and higher are required for normal breeding and reproduction (Fromm 1980). There is also species variation in the pH requirement. Levels of ammonia, CO_2, and organic acids are all important for proper pH maintenance (Moe 1992).

Salinity, Alkalinity, and Hardness

The total amount of solid materials dissolved in the water is important. Fish need specific elements to carry out vital biochemical processes and they depend on their medium of existence for the same.

Salinity. The amount of dissolved salts in the water affects the density of water and temperature requirements of some species (Tucker 1993). When transferring fish, any changes in salinity should be gradual.

Alkalinity. The alkalinity of water is a measure of acid neutralizing capacity. Bicarbonates, carbonates, borates, phosphates, and other anions contribute to alkalinity, which is expressed by millequivalents per liter (mEq/L). Adequate alkalinity (0.2-10 mEq/L; sea water 2.5 mEq/L) ensures buffering of acid metals and proper functioning of bio filters (Moe 1992; Tucker 1993).

Hardness. Hardness is the measure of the mineral content (primarily calcium, magnesium, and other divalent cations). Water can be very hard to very soft depending on the levels of dissolved minerals (Moe 1992). Appropriate hardness may decrease stress, toxicity of dissolved metals, and ammonia (Tucker 1993).

Different species vary in their requirement of hardness, pH, and salinity.

Dissolved Oxygen (DO)

To maintain healthy fish, DO should be near saturation at any temperature and salinity. Oxygen is diffused into water by various means of aeration: agitation, liquid oxygen, air diffusers (using air compressors and blowers), U-tubes, air stones, and air lifts. A decrease in DO represents a sign of stress in fish. The amount of oxygen a fish requires depends on its life stage, species, size, as well as on the temperature of the water (Tomasso 1993; Tucker 1993). A flow rate of 0.7×10^{-3} sec^{d} assures saturation. The U.S. Environmental Protection Agency (EPA 1976) has set 5 mg/L as the minimum DO concentration for optimal fish health.

Nitrogen

Nitrogen is present in water as gas, nitrite, nitrate, and ammonia. Ammonia is the most toxic inorganic nitrogen produced by fish and by the heterotrophic bacteria of biological filters. EPA considers 0.02 mg/L of un-ionized ammonia as safe (EPA 1991). In recirculating water systems, nitrite toxicity can occur with improper biological filters (Schreck and Moyle 1990). Nitrite is formed in nitrification and denitrification processes and causes methemoglobinemia, and ultimately, hypoxia (Williams and Eddy 1986). Excess ammonia and nitrite levels are primarily responsible for "new tank syndrome" in fish (Moe 1992).

Artificial Sea Water

Many researchers need to purchase (and reconstitute) or prepare artificial seawater to meet their marine fish needs. Care should be taken to ensure that all essential elements are present and that the solution is properly mixed and stored (Spotte 1979). Water can be pretreated to reduce bacteria and remove disinfectants and algicides (Stoskopf 1993c).

Shipping

Following procurement either from the field or a commercial source, the water quality during transport must be taken into account. In general, when transporting fish, cooling the water tends to decrease the metabolic rate of the animal and thus decrease the amount of ammonia excreted into the water. In addition, lower temperatures reduce the requirement for oxygen.

The specimens should be taken off food for 2-3 days prior to transport, so that they will void their digestive tracts and not foul their shipping water. Depending on the species and length of time in transit, this fasting period will usually fall between 1 and 5 days, with two being common. Fish excreta lowers pH and affects the health of the fish.

The appropriate number of boxes and heavy polyethylene bags, depending on the size, compatibility of the fishes, and the length of their transport should be used. Small 3 cm long schooling fish, for example, could go together in one large bag while larger or more territorial fishes may need to be bagged separately. As a rough guide, one box can hold about fifty 2 cm long fish, or five 8 cm long fish, or one 25 cm long fish. Obviously, different species have different requirements, so good judgment is important. If in doubt, one should lean toward packing conservatively. Most fish shipping containers are of a standard size, 15" x 15" x 7" high. They can be ordered from a variety of sources (see Table 1).

With all but the very largest species, the best way to ship fish is in a plastic bag filled with water and oxygen and packed into a styrofoam insulated container. Special purpose square-bottomed bags should be used (see Table 1) as fish get trapped in the seams of conventional bags available in the laboratory. The general procedure is to fill the transport bag about half full with the fish' s original water of permanent residence, inflate the rest of the bag with oxygen or compressed air, and tie off with a rubber band so that no oxygen or water can escape. The bag should be fully inflated (unless fish are being shipped by air cargo), so that it is tight, almost like a balloon. At least one more bag should be put over the first bag and also secured by a rubber band. Special precautions should be taken when spiny fishes are transported as they may puncture the bag. Newspapers between the inner and outer bags may help. Once the bags are prepared, they can then be closely packed flat in the styrofoam fish box, which can be put inside a larger plastic bag, placed in the cardboard outer box, and then sealed. A styrofoam box can be placed on a frozen ice block while shipping

coldwater species. A good conservative packing job should maintain fish for 12 to 24 hours. Such containers can be sent by air freight and should be appropriately labeled. Multipurpose transport boxes commonly used by the Aquarium of the Americas are good for 48-72 hour transports (Schaeffer and others 1992). If possible, it is advisable to limit the transport time to under 24 hours by express shipping.

Fish can also be transported locally in bags or coolers (using appropriate sizes for the species) with air or oxygen bubbled through the water. State agencies such as natural resource departments frequently have transport vehicles adapted for delivering fish. Occasionally fish may be lightly tranquilized for transport with an anesthetic such as MS-222 (Brown 1993). The key to successful transport is to keep transport time to a minimum and reduce stress from handling, crowding, changes in temperature, low oxygen, and elevated ammonia.

Acclimation

Acclimation of new fish should ideally begin before they arrive. It is important to know as much about the quality of the water from which the fish are coming--temperature, salinity, pH, hardness--so that the laboratory environment can be adjusted to match these parameters. If need be, once the fish have settled in, these parameters can be changed.

Once fish are received in the laboratory they should be housed in quarantine tanks that have the correct temperature and salinity for that species. Fish in transport bags should be acclimated by floating the bags in the tanks until the water temperature in the bag is the same as the tank water temperature. The bags can be secured to the tank using clamps so that they can be opened to aerate the water during this time. Samples of the bag water should be evaluated for pH and ammonia to determine under what conditions the fish have been transported. Handling the animals as little as possible and keeping the lighting low will help reduce stress.

Newly arrived fish may also be acclimated by slowly transferring water into the transport bag or container from the new system. Aeration should be provided at this time. The water transfer should take place between 10 and 30 minutes, depending on how far apart the water parameters are between shipping water and system water. Acclimation can be considered complete when these measurable parameters are the same (or at least similar).

After the temperature in the bags have equilibrated with the tank water (approximately 30 minutes), the fish can be gently netted and released into the tanks, without the transport water. Prior to releasing the fish, it is advisable to conduct a rapid physical examination on several individuals. Fish can be gently held and examined for any external lesions and then some mucus can be scraped from the skin. A gill biopsy can also be taken. Samples should be examined for parasites. If possible, a full necropsy examination should be conducted on several fish to ascertain the general health of the population (Reimschuessel 1993).

As a group, the fish should be handled as little as possible, using only a smooth container such as a glass beaker or a fine mesh net. Situations where fish have to be chased around in order to be caught can create stress. With a little forethought, a quick trap can usually be made with a couple of hand nets or a beaker.

Quarantine

Ideally all fish should go through a 30-day quarantine period. This is especially important when the system into which the new fish will go already holds healthy populations. Having separate tanks with separate equipment and tools is important to avoid any transfer of disease. It is important that the quarantine systems should be separated from the holding systems with no cross-contamination from splashing, back-washing filtration systems, or aerosol infection. All siphoning or tank cleaning equipment and nets should be uniquely dedicated to each tank. During the quarantine period fish should be examined for signs of disease or parasitism. Signs of an unhealthy or stressed fish include wobbly swimming, severe undulations, problems with maintaining buoyancy, pale color, and folded fins. Treatments for parasites may be required during this time, especially for wild-caught animals. The CCAC *Guide* (CCAC 1984) contains a chapter on fish that can help in developing facility protocols. Tropical marine fishes are vulnerable to ectoparasites, and are most often treated with copper sulfate at 0.2 mg/L for a period of 21 days. Freshwater fish generally need less proactive measures than tropical marine fish, so often a 30-day observation period will suffice (Whitaker and others 1994). If no disease manifests, the fish can be moved to the main system.

Breeding

Fish breeding protocols vary significantly among species. Small species extensively bred in the laboratory include Japanese medaka, fathead minnow, and zebrafish. The methods for producing transgenic fish for research continue to be developed (Yamamota 1975; Chen and others 1995).

Guidelines for raising fish in ponds can be developed from current aquaculture procedures (Boyd and Lichtkoppler 1979; Wheaton 1979; Piper and others 1982). When developing research ponds, several factors need to be considered including species requirements, land availability, incoming water (freshwater streams, well water, brackish or saltwater sources), and drainage. Discharge, especially for research protocols, must be in accordance with federal (EPA and USDA), state, and local regulations.

Research Laboratories

Guidelines developed for the research laboratory may be similar to those developed for a centralized facility. All incoming animals should be quarantined and acclimated before use in experiments. For toxicology and carcinogenicity research, the exposure route must be well evaluated. For aqueous exposures, tests may be conducted in static, static-renewal, or flow-through systems. The choice of system depends on the toxicant, the test species, the compound being tested, and available resources (EPA 1989,1991). In general, flow-through systems are preferable because they cause fewer fluctuations in water quality. Commercial flow-through toxicant delivery systems, however, are expensive and complex. Static systems have been widely used in research, especially with early life stages of small species such as the fathead minnow.

For toxicology and carcinogenicity research, protocols must be developed to deal with the discharge water depending on the toxicant used. This is less of a problem if the toxicant is administered by another route (such as orally or by gavage or injection). For other types of research, the protocols will vary greatly depending on the species and experimental design. For example, in vitro studies using fish tissues mostly require maintenance and sacrifice of the experimental fish. On the other hand, studies measuring physiological parameters may require surgical manipulations or catheterizations. Such procedures must be developed on a case-by-case basis.

DANGEROUS AQUATIC ANIMALS AND SAFETY CONSIDERATIONS

The key to human safety when working with any animal is a combination of awareness and common sense. Even the smallest of creatures is equipped with defense mechanisms of some sort. In addition, workers may be exposed to diseases, illnesses, and infestations that may be transmitted from and to the animals. By using proper precautions, the risk of zoonotic infection can be greatly minimized.

Dangerous Aquatic Animals

Aquatic animals have evolved numerous mechanisms for self-preservation. While some inflict injury through trauma, others are capable of delivering venom or a severe electric shock. Avoiding injury of both worker and animal requires proper handling and feeding techniques, special equipment, and a basic understanding of the unique habits of each species. Animal housing should be designed for safety and ease of use. For example, an overhead hoist and specially designed stretcher reduces the chance that large sharks will fracture their extensive liver or injure personnel when restrained. Emergency procedures must be in place and practiced on a routine basis so that all personnel are comfortable with managing an injury. Finally, at least 2 people should be present when working with animals capable of inflicting a life-threatening wound.

Traumatogenic Animals

Traumatogenic animals cause injury via a bite, sting, electric shock, puncture, or other physical mechanism. Although the list is extensive, examples include sharks, rays, barracudas, moray eels, sawfish, large groupers, piranhas, and surgeon fish. In many cases, secondary bacterial infection (Table 3) presents a greater risk to the recipient than the initial trauma. Poor hygiene and husbandry practices augment the likelihood that a serious infection will occur. All wounds, no matter how small, should be cleansed thoroughly with a disinfectant. If any question exists as to the extent of an injury or risk of secondary infection a qualified physician should be consulted.

Venomous Fish

Many venomous fish exist in both the cartilaginous (elasmobranchs) and bony (teleosts) fish groups (Table 2). Most of these venomous animals use spines capable of inoculating their victim with toxin. Although many of the teleosts inject their venom, the elasmobranchs (stingrays) carry a spine at the base of the tail that has an integumentary sheath that contains the toxin. Every attempt must be made to remove this sheath as well as the spine from the wound by various means (Russell and others 1958). Venomous fish stings should be treated immediately by soaking the wound in very hot water for up to 90 minutes, which denatures the venom's proteins (Halstead and others 1990). Venom associated with the integumentary sheath of the spine of marine rays is capable of producing significant cardiovascular effects including irreversible cardiac standstill (Russell 1957). Secondary bacterial infections often present a more serious problem for people than the toxin delivered (Table 3).

Electrogenic Animals

Electrogenic fish, of which there about 250 species, possess a specialized organ that can generate very high voltages when needed for protection or to stun prey. Proper equipment such as rubber-soled shoes and rubber gloves provides some protection against shock when handling these animals. Additionally, these animals can be agitated, causing them to produce a series of shocks leading to electrical discharge, which makes them safer to handle. Examples of electrogenic fishes include electric eels (Electrophoridae), knifefish (Notop-teridae), and catfish (Malapteruridae) in freshwater and electric rays (Torpedinidae) in saltwater.

Zoonoses

Aquatic animals live immersed within an environment of potential pathogens. The presence of microorganisms alone is a danger to caretakers. In closed systems, however, the concentration of microorganisms may be amplified increasing the risk of human infection. Typically, bacteria associated with lesions are gram-negative organisms such as *Aeromonas hydrophila* in freshwater and *Vibrio spp.* in saltwater. Other organisms can be acquired from aquatic animals (Table 3).

Once exposed, infection or disease will occur depending on the virulence of the organism and the susceptibility of the host (Geraci 1991). Following several commonsense guidelines can minimize the likelihood of a serious illness. First, and most important, is the practice of proper hygiene. Hands should always be washed with an antimicrobial soap after handling animals or working in their environment. Second, any open wounds should be covered to prevent inoculation. Third, immunosuppressed individuals should avoid exposure to potential pathogens. Fourth, ill employees should not come in contact with animals or their environment. Finally, if an injury does occur while handling an animal or working in its environment, proper first aid must be applied.

Working with all animals presents some level of danger from injury or zoonotic infection. The likelihood that an injury or infection will occur is dependent upon the individual's ability, proper hygiene, and common sense. Therefore, it is essential to adopt proper husbandry practices, use equipment designed for the specific task, train inexperienced personnel, and develop emergency protocols tailored to the animals being used.

EXAMPLES OF USES OF SPECIES IN BIOMEDICAL RESEARCH

Fish have been used in biomedical research for many years (Klontz 1971; Wolke 1984; Powers 1989). With their diverse sizes and their myriad of anatomical variations, fish offer the scientist opportunities to explore novel organs and structures. These studies can have profound implications for understanding mammalian biology and physiology. For example, one of the first investigations demonstrating the role of renal tubular secretion in the excretion of xenobiotics was accomplished using the aglomerular toadfish (Marshall and Graffiin 1982). Until then it was almost heresy to suggest that substances appearing in the urine had come from anything but glomerular filtration. More recently, nephron neogenesis following toxicant-induced injury, not found in mammals, has been demonstrated in goldfish kidneys (Reimschuessel and others 1990).

Other specialized features in fish of interest to biomedical researchers include antifreeze-like molecules in the blood of arctic species (Eastman and DeVries 1986), electrical activity in muscles of the electric eel (Meszler

and others 1974; Fendler and others 1993), survival of dehydration in the African lung fish (Sawyer and others 1982), and copper accumulation in white perch (Frazier 1984). Fish are also extensively studied as models for research on aging (Anonymous 1991; Patnaik and others 1994), vision (Djamgoz and Wagner 1992), locomotion in cells (Lee and others 1993), and leukemia (Mulcahy 1992). Species are also evaluated for pharmacologically active compounds such as Indian catfish venom (Auddy and others 1994), and angiogenic inhibitors and antineoplastic agents in shark tissues (Snodgrass and others 1976; Pettit and Ode 1977; Oikawa and others 1990). Fish are also studied as indicators of environmental pollution (McKone and Daniels 1991) using parameters such as neoplasia (Black and others 1982; Baumann and others 1987), and immunological function (Anderson 1990; Blazer and others 1994; Muhvich and others 1995).

Small species of fish have been used in many studies because their size allows large numbers to be kept in a limited space and their short life cycles provide the opportunity to examine multiple generations (DeVita 1984; May and others 1987a,b). Fish have also been used to investigate carci-nogenicity and toxicity of various compounds (Iwan and Cella 1981; Ishikawa and others 1984; EPA 1989, 1991). Japanese medaka and zebrafish transgenic specimens are being used to evaluate the roles of multiple genes in development (Streisinger and others 1981; Brenner and others 1993; Chen and others 1995)

The Armed Forces Institute of Pathology (AFIP) in A *Handbook: Animal Models of Human Disease* (AFIP 1989) lists the following species as models: multiple schwannomas of bicolor damselfish, type I diabetes in carp, DNA damage in the Amazon molly, Wilson's disease in the white perch, hepatocellular carcinoma in rainbow trout (Ayres 1971), and malignant melanoma in platy/swordtail hybrids.

Methods for Fish Biology (Schreck and Moyle 1990) presents an excellent overview of the concept and design of research methods employing fish including field experiments, fish genetics, systematics, and taxonomic methods using morphology and electrophoresis, chromosome analysis, histology, anesthesia, surgery, and hematology. It also includes specific areas of study on respirometry, growth, bioenergetics, the nervous system, stress and acclimation, aquatic toxicology, reproduction, behavior, antecology (the study of single-species ecology), community ecology, as well as a section on maintaining fish for research and teaching.

ANESTHESIA, ANALGESIA, AND EUTHANASIA

Anesthetics, when used in a judicious and appropriate manner, may provide great benefit in the relief of pain, which helps both in maintaining and handling fish. With increasing anesthetic concentrations, sedation, immobility, loss of equilibrium, and loss of consciousness can be achieved in a controlled fashion allowing for a variety of procedures to be safely carried out, both for the animal as well as the handler. Anesthetics are used in teleosts and elasmobranchs to perform surgical and diagnostic procedures, and to facilitate capture, handling, and transport.

A good anesthetic should provide predictable results including effective analgesia, good immobilization, and rapid induction and recovery, while allowing for a wide margin of safety (Brown 1993). Although pain and suffering in fish are poorly understood, it is clear that the proper use of an agent can minimize the stress experienced by a fish and therefore prevent the cascade of physiological and biochemical changes that result from a fright and flight situation (Davis 1992; Iwana 1992). Avoiding such an internal upheaval within a fish is key in preventing the disruption of osmoregulation, loss of immune function, and decreased reproduction.

While topical and local anesthetics have been used on occasion in fish, general anesthetics are more commonly applied. The majority of chemicals used as general anesthetics mix well with water and allow for minimal restraint once the fish have been placed into a designated induction pool. The water quality of this chamber should match closely that in which the fish have been kept. Of particular interest are salinity, hardiness, pH, dissolved oxygen, and temperature. The easiest way to achieve this is to remove water directly from the fish's environment and then add anesthetic. Under other circumstances, it may be desirable to add anesthetic directly to the fish environment. This is done by achieving the appropriate anesthetic concentrations in the given amount of water. This is particularly advantageous when large numbers of animals are to be sedated for movement from one system to another.

The stages of anesthesia (Table 4) are similar to those observed in other animals. The dosage used depends on the species, as well as the metabolic ability and overall health of the individual fish. It may be helpful to use conservative doses on I or 2 representative animals, adding anesthetic as needed to move quickly through the excitement phase where injury is most likely to occur.

Anesthesia is maintained by continued exposure to varying concentrations of the agent. Intermittent administration of the anesthetic solution over the gills may be done by simply using 60 cm^3 syringes. Alternatively a drip system can be created using 2 bags connected by IV tubing and a 3-way stopcock for mixing. More advanced anesthetic machines can be easily constructed that allow for a continuous flow of well-oxygenated and titrated anesthetic to the animals.

Monitoring the depth of anesthesia becomes increasingly difficult as the fish loses its equilibrium, stops swimming, fails to respond to deep pressure, and subsequently ceases any opercular activity. Slow and steady opercular movements without response to physical stimuli are desirable. On larger animals, an electrocardiogram (ECG) may provide valuable information about heart rate as well as atrial and ventricular activity. With hypoxia for instance, the T-wave amplitude will increase as an irregular rhythm is observed (Harms and Bakal 1994).

For recovery, the animals can be placed in a well-oxygenated, anesthetic-free environment. Jaw tone will return before opercular activity (Harms and Bakal 1994). Propelling the fish head first through the water will force water through the mouth and over the gills effectively removing the drug while oxygenating the fish.

The list of potential fish anesthetics is long. When deciding upon a particular chemical one must take into account its intended use, availability, cost, legal issues, and personal preference. To date only Finquel (tricaine methanesulfonate) has been approved for use in food fish by the Food and Drug Administration. Even so, its label prohibits the immediate release or consumption of fish within 21 days of treatment. Carbon dioxide and bicarbonate have also been used as anesthetics in food fish and although they are not labeled for this use, they are food additives that are generally recognized as safe (Summerfelt and Smith 1990).

Tricaine Methanesulfonate (Finquel, MS-222)

This fine white crystal is highly soluble in water and is related to Novocain, procaine, and benzocaine. It is absorbed rapidly via gill diffusion or by coupling to specific enzyme systems (Summerfelt and Smith 1990). In teleosts, MS-222 is biotransformed in the liver and probably the kidney (Harms and Bakal 1994). The metabolic transformation of tricaine in elasmobranchs, such as the spiny dogfish, however, appears minimal (Dunn 1990). A stock solution of 10 g/L of carbon filtered fresh or distilled water may be prepared. This solution may be stored at room temperature in opaque plastic or brown glass containers. With exposure to sunlight the solution will turn brown.

The anesthetic dosage of MS-222 in fish ranges from 50-200 mg/L. Like many of the anesthetics, there exists a wide range of species sensitivity. Aeration should be provided in the anesthetic solution as hypoxia is a potential side affect. Because of its acidic nature, a solution of 100 mg/L has a pH of 5.0 in very soft water. Buffering the stock solution is not feasible due to formation of a white precipitate. Adjustments must therefore be made after making the appropriate dilution. The pH of seawater does not appear to be significantly affected by the addition of stock solution.

Quinaldine Sulfate (2-Methylquinoline Sulfate) and Quinaldine (2-Methylquinoline)

Quinaldine sulfate is a highly soluble light yellow powder that has a wider margin of safety than tricaine. It is not metabolized by fish and is excreted unchanged (Harms and Bakal 1994). Unlike its cousin Quinaldine, which is a slightly water-soluble, oily liquid, the salt Quinaldine sulfate has no odor. Both are irritating to mucous membranes. Like tricaine, Quinaldine depresses sensory centers of the central nervous system. Because it is lipid soluble, Quinaldine accumulates preferentially in the brain (Summerfelt and Smith 1990). Quinaldine and stock solutions of Quinaldine sulfate must be stored in tightly sealed containers that protect them from light.

Fish anesthetized with either Quinaldine sulfate or Quinaldine maintain a response to pressure that dissipates after approximately 20 seconds of contact. In addition, their analgesic qualities are questionable making them a poor choice for most surgical procedures. Quinaldine doses range from 5-12 mg/L in salmonids, and 2.5-30 mg/L or higher for warm water fish. Tilapia are insensitive to the effects of Quinaldine requiring concentrations of 50-1000 mg/L to reach an appropriate plane of anesthesia (Summerfelt and Smith 1990).

Although Quinaldine sulfate acidifies the water like tricaine, induction and recovery times are faster than experienced with Quinaldine. Effective concentrations are 25 mg/ L for salmonids and 15-60 mg/L for warm water species. While carp are sensitive to this drug large-mouth bass are less so (Stoskopf 1993d).

Benzocaine (Ethyl Aminobenzoate) and Benzocaine Hydrochloride

Benzocaine is a highly insoluble powder that must be dissolved in either ethanol or acetone. A stock solution of 100 g/L is generally prepared. A water soluble form of the drug, benzocaine hydrochloride, is available. Like tricaine, buffering benzocaine hydrochloride working solutions may be necessary. Both solutions must be stored in light protected, airtight containers. Because benzocaine is hydrolyzed to para-aminobenzoic acid, this anesthetic should be avoided in animals receiving sulfonamides (Brown 1993). Concentrations typically range from 25-200 mg/L.

Metomidate (Marinil)

Metomidate is an imidazole-based nonbarbituate hypnotic agent that has been used in fish. The drug was initially thought to eliminate the stress response as elevated cortisol peaks were absent in anesthetized patients. This appears not to be the case as it is thought that metomidate suppresses cortisol synthesis through the suppression of 11-B-hydroxy-lation of cholesterol (Stoskopf 1993d).

Doses for transport range from 0.06-0.20 mg/L. Anesthesia is achieved in most fish using 2.5-5.0 mg/L. Induction is rapid although recovery may be prolonged in animals exposed to the initial dose for long periods of time.

2-Phenoxyethanol

2-Phenoxyethanol is an oily liquid with slight solubility that is added directly to the water. Anesthetic concentrations range from 0.08--0.5 ml/L. Unfortunately, this chemical has a narrow margin of safety and sublethal exposure may cause liver and kidney damage (Summerfelt and Smith 1990). For these reasons, 2-phenoxyethanol is not a desirable anesthetic for use in fish.

Ketamine Hydrochloride (HCL) and Ketamine HCL/Xylazine

Ketamine provides excellent analgesia and anesthesia in teleosts when injected intramuscularly at a dose of 60-80 mg/ kg (Williams and others 1988). Because of the large volumes associated with this dose, ketamine may be lyophilized for reconstitution in smaller volumes. Lower doses of 12-20 mg/kg are effective in carcharhinid sharks. Although species specific, induction generally takes 10-20 minutes and provides 10-20 minutes of surgical anesthesia. A modified spear gun may be made to carry darts swiftly through the water allowing the acquisition of select individuals from a large body of water. Bruising or scale loss may occur at the injection site. If available, tricaine, benzocaine, or Quinaldine sulfate are easier, quicker, and safer to use than ketamine.

Ketamine HCL (12 mg/kg) when mixed with xylazine (6 rog/kg) provides an excellent anesthetic cocktail for sharks. Muscle spasms that occur with ketamine alone are no longer observed with this combination (Stoskopf 1993e). Doxapram HCL and yohimbine given intravenously are effective reversal agents.

Carbon Dioxide

Carbon dioxide has been used as a fish anesthetic for many years. It is readily available in the form of Alka-Seltzer™, which when added to water at a rate of 3 tablets per 400 ml produces an equilibrium of carbon dioxide, bicarbonate, and carbonic acid. Despite its ease of use and lack of toxic residues this method of anesthesia is stressful as it alters blood gases and acid-base balance within the animal (Iwama and others 1988). Alternative anesthetics should be used.

Electroanesthesia

There is limited research in this area. Electroanesthesia provides a very rapid tool to immobilize fish for minor procedures. It is most useful where large numbers of animals need to be collected quickly or the use of chemical agents may interfere with the research being done. Although the technique is considered safe, occasional trauma or cardiac fibrillation may be seen.

Euthanasia

Several methods of euthanasia have been used in fish including hypothermia, electrocution, overdosing with tricaine or carbon dioxide, and a sharp blow to the head. Of these, tricaine administered at 500 mg/L is most desirable as it does not alter blood cortisol, catecholamine, or glucose levels commonly associated with stress (Harrell 1992). Where chemicals may alter experimental data, cranial concussion followed by some other physical method may be employed (Andrews 1993).

For fish, the *Report of the A VMA Panel on Euthanasia* (AVMA 1993) lists as acceptable tricaine methanesulfonate, benzocaine, and barbiturates, and as conditionally acceptable, stunning by blow and decapitation, and decapitation.

IACUC Protocol Review

When reviewing protocols involving fish, it is of utmost importance that the IACUC have the benefit of direct knowledge and experience in the use of fish in the laboratory or in the field and with the particular species (or related species) concerned. The committee should feel free to use outside reviewers for proposed projects with which they have limited experience. Another critical part of the IACUC review is to be certain that all laws and regulations are followed. The acquisition offish may require licenses and permits, and failure to obtain these could adversely impact the study and the institution.

Capture techniques, such as the use of seines and traps, gill netting, ichthyocides, electrofishing, and hooks or spears must be carefully reviewed as to their necessity and the possibility of capture distress (ASIH 1987). The fish habitat should be as undisturbed as possible to support the concept of habitat conservation. Restraint and handling in the particular proposal should look to limit the amount of time of handling, especially with large or venomous fish. A team approach when working with these fish should be encouraged or required depending on the particular circumstances. In any case, the investigator (or investigators) should be experienced in handling these species or have a plan to receive training prior to the initiation of the protocol. In general, a protocol should not be approved by the IACUC unless the investigator has appropriate training in the particular handling, care, and use of the species proposed. Prolonged restraint should be avoided (see above, under Anesthesia, Analgesia, and Euthanasia).

Marking fish for field study is important to analyze their movements and population biology (Schreck and Moyle 1990; ASIH 1987). One must consider the effect of the marking on fish behavior and health. There are a variety of identification methods, including fin-clipping, electrocauterization or freeze branding (under a local or a general anesthetic), tattooing, acrylic paint injections, tagging, radiotelemetry, and radioisotope injections. Tagging is used most frequently and has been widely investigated.

Release of feral fish back into the wild should not be approved unless it is in accord with all federal, state, and local requirements. The fish must be in excellent health, able to function in the new environment, released only within its native range of distribution, and unlikely to spread any pathogenic agents. In the event that the fish must be euthanized, it is essential to use acceptable criteria to ensure death prior to disposal (ASIH 1987).

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TABLE I Fish suppliers and other resources

Saltwater fish vendors & collectors

Pacific/Indian Ocean Tropicals

Avista International, Inc.
Falls Church, VA
Tel: 703/573-4136

Cortez Handcaught Marines, Inc
Inglewood, CA
Tel: 310/215-0303

Quality Marine
Los Angeles, CA
Tel: 310/645-1107

Atlantic Tropicals

Captains' Marine
Key West, FL
Tel: 305/294-7683

Dynasty Marine
Marathon, FL
Tel: 305/743-7666

Gulf Specimen Marine Laboratories, Inc.
Panacea, FL
904/984-5297

Cold Marine

Marine Biological Laboratory
Woods Hole, MA
Tel: 508/548-3705, ext. 375

Pacific Bio-Marine Labs, Inc.
Venice, CA
Tel: 310/677-1056

Sea Life Supply
Sand City, CA
Tel: 408/394-0828

Laboratory supply (fish)

Carolina Biological Supply Co.
Burlington, NC
Tel: 919/584-0381

Ward's Biology
Rochester, NY
Tel: 800/962-2660

Fish shipping boxes

J.V. Industries
Hialeah, FL
Tel: 305/885-4666

Lifelike Plastics
Miami, FL
Tel: 305/835-0646

Fish shipping bags

Seal-Tite Plastic Packaging Co.
Miami, FL
Tel: 305/264-9015

Saltwater sources

Forty Fathoms. Marine Enterprises, Inc.
8755 Mylander Lane
Towson MD 21204
Tel: 301/321-1189

H.W. Marine Mix
Hawaiian Marine Import, Inc.
PO Box 218687
Houston, TX 77218
Tel: 713/492-1189

Instant Ocean. Aquarium Systems
8141 Tyler Blvd.
Mentor, OH 44060
Tel: 216/255-1997

Jungle Ocean 50. Jungle Laboratories Corp.
Box 630
Cibolo TX 78208
Tel: 800/327-2200 or 512/658-3503

Kahl Sea Salt
Kahl Scientific Instrument Corporation
PO Box 1166
El Cajon, CA 92202-1166
Tel: 619/444-2158 or 5944

Marine Environment.
Import Associates, Inc.
P.O. Box 16350
San Francisco, CA 94116
Tel: 415/591-2200

Rila Marine Mix. Rila Products
P.O. Box 114
Teaneck NJ 07666
Tel: 201/836-0855

National aquaculture suppliers

are listed in:
Aquaculture Magazine Buyer's Guide
PO Box 2329
Asheville, NC 28802
Tel: 704/254-7334

Federal Wildlife Permit Office

U.S. Fish & Wildlife Service
4401 N. Fairfax Dr.
Arlington, VA 22203
Tel: 800/358-2104

TABLE 2 Venomous/spiny fish

Elasmobranchs stingrays or whip rays devil rays or mantas eagle rays or bat rays cownose rays round stingrays *Freshwater rays* S. American river rays Spiny Dogfish Ratfish or Elephantfish	*Dasyatidae* *Mobulidae* *Myliobatidae* *Rhinopteridae* *Urolophidae* *Potamotrygonidae spp.* *Squalidae* *Chimaeridae*	**Teleosts** Catfish Ariidae, Clariidae, Plotosidae Weeverfish Trachinidae Scorpionfish Scorpaenidae includes 60 genera and over 300 species Scorpionfish Lionfish Stonefish Sculpin Rockfish Toadfish Surgeonfish[1] Dragonets Rabbitfish Stargazers Leatherbacks[2]	Ariidae, Clariidae, Plotosidae Trachinidae Scorpaenidae Batrachoididae Acanthuridae Callionymidae Siganidae Uranoscopidae Carangidae

[1]Some surgeonfish have venomous spines at the base of the tail, others do not, but all can inflict a wound.
[2]*Scomberoides sanctipetri* is the only carangid (which includes jacks, scads, and pompanos) known to have venomous spines (Halstead and others 1990).

TABLE 3 Aquatic microorganisms associated with zoonotic infections

Aeromonas hydrophila Atypical Mycobacteria *M. fortuitum* *M. marinum* *M. chelonia* Campylobacter spp. *Edwardsiella tarda* Enteropathogenic *E. coli* Enterotoxic *E. coli* Erysipelothrix spp. *Legionella pneumophila* Pseudomonas spp. Salmonella spp. *Vibrio cholera* *Vibrio parahemolyticus* *Vibrio vulnificus* *Vibrio fluvialis* *Yersinia enterocolitica*

TABLE 4 Stages of anesthesia

Stage I: Induction Erratic swimming, excitement phase, some loss of equilibrium, disorientation, increased respiration, some loss of tactile response, reduced activity
Stage II: Sedation Loss of equilibrium, slow swimming without direction,decreased respiration
Stage III: Anesthesia 1. Complete loss of equilibrium, swimming and respiratory activity slowed, still responsive to stimuli 2. Surgical plane, unable to swim, respiration becomes shallow, no response to stimuli 3. Cessation of opercular movements
Stage IV: Pre-Mortem Spasmodic over-distention of opercules, cardiac failure

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A Virtual Tour of the *Guide* for Zebrafish Users

Monte Matthews, Bill Trevarrow, PhD, and Jennifer Matthews, DVM, PhD

PHS-funded and AAALAC-accredit- ed facilities are required to use the *Guide* as the basis for setting up a zebrafish care and use program. The authors describe how they accomplished this task at the University of Oregon Zebrafish Facility.

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Although fish have long been used in bio- medical research and testing, only in the last 30 years has the zebrafish *(Danio rerio)* become an important research tool'. The explosion of developmental biology, neu- robiology, and genetics research[2-4], as well as environmental science, teratology, carcinogenicity testing, and reproductive and behavioral studies[3-6], have contributed to the rise in popularity of the laboratory zebrafish.

Several zebrafish qualities contribute to their suitability as models for biomedical research. First, they are easy to maintain in large numbers, readily reproducing under laboratory conditions. Second, adult fish can be subjected to mutagenesis and hap- loid embryos screened for mutations in the first generation. Third, the zebrafish embryo has few cells relative to other vertebrates, making it a "simple" model for more complex vertebrates; moreover, the embryo is transparent and develops very rapidly and externally, permitting ready observation of the events involved in differentiation of tissues such as the nervous system. Fourth, direct access to the developing embryos permits such experimental manipulations as introducing foreign genetic material and labeling of cells. Finally, their small size allows the large numbers of zebrafish required for genetics studies to be easily maintained[7].

Many of the institutions using zebrafish for research, testing, or teaching are funded by the Public Health Service (PHS) and/or accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). Therefore, these institutions should use the *Guide for the Care and Use of Laboratory Animals*[8] *(Guide)* as a basis for designing, implementing, and evaluating the program for zebrafish care and use.

Because PHS *Policy on the Humane Care and Use of Laboratory Animals*[9] *(PHS Policy)* defines animal as "Any live, verte- brate animal used or intended for use in research, research training, experimenta- tion, or biological testing or for related purposes," institutions that receive PHS funds or support must have a defined pol- icy Assurance that describes the institu- tiors compliance with the PHS *Policy* and the *Guide*.

Although the *Guide* and PHS *Policy do* not provide specific guidelines for the use of zebrafish, the Office of Laboratory Animal Welfare (OLAW) states, 'Many of the principles embodied in the *Guide,* although not specifically addressing cold-blooded vertebrates, generally can be adapted to animal care and use programs for various kinds of amphibians, reptiles, and fishes[10]." Detailed descriptions of the care and use of zebrafish are clearly beyond the scope of this article, but we will demonstrate in broad terms-based on our experiences with zebrafish at the University of Oregon-how the principles in the *Guide* can be applied to the use of zebrafish in animal research programs, referring to each chapter in the *Guide,* with the exception of the physical plant descrip- tion.

The 1996 revision of the *Guide,* which emphasizes performance-based standards, allows institutions to develop and define their own goals, the methods for achieving those goals, and the means for evaluating them. This approach is particularly useful for zebrafish users who have few engineer- ing standards to follow[8].

Although zebrafish are not covered by United States Department of Agriculture (USDA) regulations, the University of Oregon FACUC has chosen to adopt a sin- gle standard of care when dealing with ani-

mals that are not covered by both the PHS *Policy* and USDA regulations. For instance, we use USDA's 12-hour rule for defining a study area rather than PHS's 24-hour rule for animal facility. Also, for painful or distressful procedures, we require the principal investigator who works with zebrafish to perform an alternatives search (USDA requirement).

Institutional Policies and Responsibilities

PHS-funded and AAALAC-accredited zebrafish facilities must have an Institutional Animal Care and Use Committee (IACUC) to oversee the animal program, facilities, and animal procedures, and to ensure that the institution's program is based on the *Guide* and PHS *Policy. The US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing Research, and Training"* form the basis of the *Guide* and can be used by IACUCs to evaluate their program and individual animal use proto- cols. For example, regarding the minimization of discomfort, distress, and pain, Principle IV states, "Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals." Because little is known about zebrafish pain, distress, and discomfort, the University of Oregon IACUC uses this principle when evaluating potentially painful or stressful procedures.

PHS Policy Section IV and Chapter I of the *Guide* describe general IACUC functions and responsibilities. The protocol review process, personnel training, and occupational health and safety (OHS) are examples of some unique applications we have developed for zebrafish users.

Protocol Review

Sections IV.C and IV.D in PHS *Policy* and Chapter I in the *Guide* describe those components that need to be addressed in the animal care and use protocol or activity. Because many of our zebrafish researchers perform identical animal use procedures, have similar reasons for using zebrafish, use a centralized zebrafish facility for husbandry and care, and have standard procedures for minimization of pain, distress, discomfort and injury, the University of Oregon has adopted a template *Zebrafish Protocol Form*[7]. This form still requires the Principal Investigator to fill out all the animal care and use procedures that are not described in the template protocol or the *Zebrafish Book*[12], and are unique to the study.

Personnel Qualifications and Training

All personnel who work with zebrafish must be adequately trained in the techniques described in the protocol or included in general zebrafish husbandry and care. Training or instruction must be made available to all researchers, technicians, students and other personnel involved in zebrafish care or use. Most training at our zebrafish facility is task-specific and oriented either to the individual or to small groups.

Occupational Health and Safety of Personnel

As required by PHS *Policy*and as stated in the *Guide,* "An occupational health and safety program must be a part of the over- all animal care and use program. The pro- gram must be consistent with federal, state, and local regulations and should focus on maintaining a safe and healthy work- place[8]" 'An OHS program should be based on the *Guide, Occupational Health and Safety in the Care and Use of Research Animals*[13], and *Biosafety in Microbiological and Biomedical Laboratories*[14]. Essential to the OHS program are hazard identification and risk assessment, personnel training, personal hygiene, facilities, procedures and monitoring, personal protection, medical evaluation, and preventive medicine.

Those who work with zebrafish at the University of Oregon are required to participate in the OHS program. As part of our training OHS program, we use a one- page handout designed specifically for those working with fish that conveys information about zoonoses, personal hygiene, and other hazards associated with animal exposure. Participation in the OHS pro- gram is linked to the animal use protocol [7].

Aside from food poisonings, the overall incidence of transmission of disease producing

Known and potential fishborne zoonoses.

***Mycobacterium* spp.**
Organisms in the genus *Mycobacterium* are nonmotile, acid-fast rods. There are multiple atypical (non-tuberculosis) species of *Mycobacterium* (*M. marinum, M. fortuitum, M. chelonae, M. abscessus*) that are recognized pathogens of laboratory zebrafish. Humans can be infected by contamination of lacerated or abraded skin with aquarium water or fish contact. A localized granulomatous nodule may form at the site of infection, most commonly on hands or fingers. The granulomas usually appear approximately six to eight weeks after exposure to the organism. They initially appear as reddish bumps (papules) that slowly enlarge into purplish nodules. The infection can spread to nearby lymph nodes. More disseminated forms of the disease are likely in immunocompromised individuals. It is possible for these species of *Mycobacterium* to cause some degree of positive reaction to the tuberculin skin test.

***Aeromonas* spp.**
Aeromonad organisms are facultative anaerobic, Gram-negative rods, which can produce septicemia in infected fish. The species most commonly isolated is *A. hydrophilia*. It is found worldwide in tropical fresh water and is considered part of the normal intestinal microflora of healthy fish. Humans infected with *Aeromonas* may show a variety of clinical signs, but the two most common syndromes are gastroenteritis and localized wound infections. Again, infections are more common and serious in the immunocompromised individual.

Other Bacteria and Protozoa
Below is a list of additional zoonotic organisms that have been documented in fish or aquarium water. Human infections are typically acquired through ingestion of contaminated water resulting in gastroenteritis symptoms or wound contamination.

Gram-negative Organisms: *Plesiomonas shigelloides, Pseudomonas fluorescens, Escherichia coli, Salmonella* spp., *Klebsiella* spp., *Edwardsiella tarda*

Gram-positive Organisms: *Streptococcus, Staphylcoccus, Clostridium, Erysipelothrix, Nocardia*

Protozoa: *Cryptosporidium*

agents from fish to humans is low. In general, humans contract fishborne disease through ingestion of infected fish tissues or aquarium water, or by contamination of lacerated or abraded skin. To minimize the possibility of exposure to one these agents, zebrafish handlers should wash their hands regularly, wear gloves, seek medical attention promptly if exposure is suspected, and remember to inform their physician that they work with fish should they become ill.

Animal Environment, Housing, and Management

Physical Environment

Microenvironment and microenvironment: The tanks or aquaria that contain the animals make up the microenvironment, which predominantly comprises water and the small air volume enclosed by the tank cover. The room housing the fish tanks constitutes the macroenvironment. There are many ways to link the micro and macroenvironments, depending on the arrangement of the laboratory and water system.

Automatic water exchange can be by either continuous or discontinuous flowthrough, or by recirculation. A flow-through system introduces clean water into the tank and discards outflow water. A recirculating water system processes the outflow water from the tanks by one or more filtration steps to remove undesirable materials and compounds and restore desirable ones. This treated water is then recalculated to the tanks. Tanks maintained by manual water changes should be fitted with small filtration units that will continually remove undesirable materials from the water.

Housing: Zebrafish are usually kept in transparent glass, Plexiglas (acrylic), or polycarbonate tanks or aquaria, permitting easy observation of the animals. Tank drains should be screened to keep fish in the tank. Ideally, the drains should be designed so that they require no cleaning until replacement. Zebrafish space requirements for physiological, behavioral, and social interactions are affected by other variables such as water quality, food and feeding regimen, size, and age, and therefore may require optimization in each facility (see below).

Zebrafish kept together for breeding should have some means to escape from more aggressive fish, in the form of either more space or plantlike materials to be used as hiding spaces. To prevent zebrafish from eating their eggs, the tank bottom should be fitted so as to make the eggs (-1.0-1.5 mm diameter) inaccessible to the fish, yet easy to collect for example, a layer of marbles, an array of closely spaced rods, mesh, or a box containing marbles or covered with mesh.

Space recommendations: Zebrafish space requirements are usually given as numbers of fish per volume of water. As schooling fish, zebrafish can be kept at fairly high densities. We use the following general guidelines. Starting with 20 eggs or embryos per 100 ml water, the 20 fish can be kept in volumes ranging from 400 ml as young larvae to 3 liters as the fish approach juvenile stage. Recommended density for growing juvenile fish and holding adults is five fish/liter. Growing fish are considered to require more space than breeding adults, which require more space than nonbreeding adults, but this requirement has not been critically tested for zebrafish. In small breeding tanks, a pair of fish can be kept overnight in 1.5 liters or as many as six fish in 2.3 liters of water.

Depending on experimental parameters, such criteria as survivability, growth rate, and fecundity can be used to assess not only the adequacy of the space being provided, but also the genetics of the fish, the food quality, frequency of feeding, and the water quality. For eggs and embryos the most appropriate criteria seem to be survival (80-95% is good) and final size obtained (1.0-1.5 cm is good) over a standard time period (0-21 days post fertilization in our facility). During the first few days fish larvae should be kept in shallow water, so that they can gulp some air to inflate their swim bladder[15]. For juvenile fish, a useful standard consists of rapid growth to a breedable adult size, with a minimum variation in size. For adult fish, lack of mortality and the ease and reproducibility of breeding success define successful husbandry.

Temperature and humidity: Zebrafish can tolerate a fairly wide temperature range. They have been kept for fairly long periods at 22-30°C (71.6-86.0°F) and can survive temperatures of 18-32°C (64.4-89.6°F). A widely used standard temperature for developmental studies is 28.5°C (or 83.3°F). Our facility is frequently kept at this temperature. Heat-shock experiments have been carried out at temperatures above 30°C (or 86°F). A gradual drop in temperature to 22-23°C (or 71.6-73.4°F) to lower the zebrafish metabolic rate is acceptable in emergencies, such as water system mechanical failures.

Water temperature can be monitored by daily thermometer readings or by electronic thermostat readings. Automatic temperature monitoring is preferable because problems can be detected immediately. Thermostats are usually used to control the water temperature, either by heating the water directly or by heating the room air. To maintain a given water temperature and compensate for evaporative cooing, one should expect to heat the air a degree or two warmer.

Ventilation: Proper ventilation for a fish tank or facility depends primarily on sufficient oxygen (O_2) supply and adequate carbon dioxide (CO_2) removal. To facilitate optimal $0_2/CO_2$ exchange, the surface area and turbulence of the water in tanks can be increased, bringing the 0_2-poor, $C0_2$-rich water to the surface so that the diffusion of the gasses is driven by concentration differences. This can be accomplished in individual fish tanks, centrally in a pump/filtration area, or both, with aeration and gas exchange equipment.

An adequate dissolved oxygen (DO) reading is 6.0 p.p.m. (mg/l). Fish that remain close to the surface of the water and appear to be gulping air are probably not getting enough 0_2. Whether a given tank contains adequate oxygen depends on many variables: number and size of fish, tank surface area, aeration in the tank,

temperature (oxygen is more soluble at lower temperatures), and rate of water exchange if there is central aeration. If pos- sible, the fish rooms should be kept at slightly positive pressures to exclude potential pathogens.

Illumination: Illumination is important for breeding and minimizing stress and dis- ease. For these purposes, standard fluores- cent lamps are sufficient. Eliminating cer- tain wavelengths may inhibit algae growth, but this is not as critical for fish health as the intensity (between 5 and 30 ft cd or 54-324 lux at the surface of the water) and the circadian light cycle (14 h light, 10 h dark). Unlike other fish, zebrafish do not require a seasonal change in their day length to bring them into a breeding state.

Fluorescent lighting is relatively inex- pensive and easy to maintain. Fixtures sim- ilar to stack lights in libraries or asymmet- rical wau-washing lights used in commer- cial displays will provide better directed light and thus better illumination levels.

Noise: Zebrafish react to loud sudden noises. Their sensitivity to various vibra- tions or sounds like talking or music is uncertain. Although they do not display any obvious reactions to such sounds, whether or not they are stressed has not been well investigated. Fish raised in a par- ticular environment may adapt to the stim- uli commonly present there. Fish raised in one environment may become stressed on being moved to an unfamiliar one. Common design considerations and man- agement decisions are usually based on human health and comfort.

Behavioral Management

Structural environment: As shallow-water schooling fish, zebrafish seem more or less indifferent to environmental enrich- ments, with the possible exceptions of such environmental irregularities that become a focus of egg-laying during mating.

Social environment: Although their schooling habits indicate they are somewhat sociable, zebrafish may not require social interactions. Indeed, zebrafish have been kept in isolation for extended periods, after which they have still bred successfully.

Activity: Species-typical behaviors include swimming, feeding, mating, and social interactions. Human interaction with fish can be stressful. Habituation to the presence of humans may reduce or eliminate the stress.

Husbandry

Food: The precise nutritional require- ments of zebrafish have not been deter- mined. There are two nutritional models for zebrafish – warm-water and cold-water commercial aquacultural fish. Neither is perfect. Zebrafish are warm-water cypri- naformes. The commercial fish with well- studied dietary requirements are cold- water cyprinaformes or rather distantly related warm-water fish.

Zebrafish are fed different foods depending on their age. Young zebrafish require small sized food items that can be swallowed whole. Larger juveniles can swallow larger items but also require more food. Newly hatched zebrafish can eat paramecia (800 μm x 80 μm), as well as a variety of prepared foods, infusoria, and rotifers. As they grow larger, zebrafish hatchlings can add to their diet larger items such as vinegar eels, microworms, or larger prepared foods. Eventually they are large enough to eat Artemia nauplii (newly hatched brine shrimp), which have a high protein content and can be hatched on demand in large numbers, but the eggs can be very expensive. Alternative foods in this size include cultured *Moina* and larger sizes of prepared foods.

Juvenile AB fish in our facility thrive on live foods, an observation that is supported by results of other investigators. Those studies indicate that some less inbred pet store zebrafish do as well on either live or prepared foods, whereas the AB fish still do better on live foods than prepared foods. Hence, facility-specific growth rate and survival tests should be used to optimize results. Adult-size fish can be fed adult pre- pared foods (tropical fish flake foods, tropical fish micropeuets, and ground trout meal) and live adult brine shrimp. Alternative live foods include *Artemia, Moina, and Drosophila* larvae.

To minimize contamination, prepared foods should be purchased in small amounts, stored only a few months, and kept frozen or refrigerated in sealed con- tainers. AU food containers should be labeled with date of production (if known), date of receipt, and date opened. Live foods should be cultured on the premises, derived from environments considered unlikely to harbor pathogens of freshwater fish (eg., brine shrimp cysts), or treated after collection to decontaminate (e.g., bleach-decapsulated brine shrimp cysts). Live foods collected directly from the wild in fresh waters should not be used as food for laboratory zebrafish.

Water: Critical for successful zebrafish maintenance are control and monitoring of water quality. An understanding of the complex chemical cycles and the many dynamic components involved can help in manipulating or controlling parameters through chemical additions and filtration processes. Among the chemical cycles are the nitrogen, carbon, oxygen, and carbon- ate and buffering cycles. These processes partially overlap and can interact exten- sively. One reason for choosing zebrafish as a model system is their broad tolerance of water conditions.

Water quality parameters can be moni- tored by hand or by instruments. Procedures can use calorimetric reagents, electrodes of various kinds, or various combinations of the two. Which parame- ters should be monitored and what they indicate are complex issues that involve a good understanding of your particular water system. Among the most useful water parameters to monitor are concentrations of ammonia, [NH_3/NH_4^+]; nitrite, [$NO2^-$] nitrate, [$NO3^-$]; calcium, [Ca_2^+]; and mag- nesium, [Mg2+1; temperature; pH; buffer- ing (or alkalinity); salinity (either as dis- solved solids or conductivity); and levels of dissolved oxygen (DO). In addition, it is sometimes important to determine the chlorine concentration, [Cl2] – as distinct from the chloride ion concentration, [Cl_2], which is a component of salinity – and the total dissolved gases (to determine whether or not the water is supersaturated).

Wedemeyer[16], Moe[17], and Spottell[18] should be consulted for a comprehensive review of water-quality parameters.

Monitoring should be sufficiently frequent to detect problems. Some parameters should be checked several times daily, others once a day, some only once a week or less. For example, mechanical systems, such as pumps, should be continuously monitored. Some water-quality parame- ters that change rapidly, like DO and salin- ity, should be monitored daily. Other para- meters, such as NH_3/NH_4^+, NO_2^-, NO_3^-, and calcium, can be monitored weekly. These monitoring frequencies will depend on each facility's set-up and experience. Several companies produce systems that can automatically monitor, log data, trigger alarms at certain thresholds, and dial-out to people who are "on call.'

As outlined above, water systems can be flowthrough or recirculating, and centralized or distributed. A flowthrough water system maintains good water quality by exchanging old water for new. Although all water systems exchange some water, recirculating water systems rely more heavily than flowthrough systems on maintaining good filtration processes. A well-operated recirculating water system will provide greater control over the fish environment and less sensitivity to external environmental variables. Centralized water systems maintain water quality using a relatively small number of pieces of equipment serving many fish tanks. More distributed systems will involve fewer tanks being served by a given set of equipment, whereas more centralized systems generally make more efficient use of labor in maintenance and can cost less. Because centralized water systems circulate essentially the same water through many or all tanks, a water quality parameter affecting many tanks can be measured with one test in a single location. By contrast, in a distributed water system, the test would have to be repeated for each independently regulated body of water. On the other hand, distributed water systems provide greater insecurity because water is not shared among as many tanks. Even though water in a recirculating system is typically exposed to UV light to kill bacteria in the water, this treatment should not be expect- ed to provide 100% protection against any organism. The choice of which water-quall- ity parameters to monitor depends on the system design (e.g., in a flow-through water system using dechlorinated tap water, frequent monitoring of chlorine levels is essential).

Sanitation: A good sanitation program prevents buildup of waste products and potential cross-contamination between tanks. Holding and breeding tanks, and their tops, can be washed with disinfectant or sterilized by autoclaving. Racks and rooms can also be sanitized periodically as necessary. Nets used with fish can be sterilized by autoclaving or disinfected by bleaching. Tank surfaces need regular cleaning to permit easy viewing of the fish.

Waste disposal: Tank wastes, including uneaten food, fish feces, and dead, decayed fish, must be removed from the tank by hand (siphoning), by the tank's filter, or by a centralized (room-scale or rack-scale) water system filter. Filters, if used, must also be cleaned to remove filtered material from the water system. Tank wastes, as well as euthanized fish and wastewater, will usually be disposed of through municipal sewer lines. Important considerations include preventing contamination of the local environment either with disease pathogens (perhaps being used intentionally in the laboratory) or with foreign organisms-recalling that zebrafish are native to water- sheds in and around India and Myanmar.

Pest control: Pests most often encoun- tered in fish facilities are arthropods: roaches, silverfish, and lice. Control con- sists predominantly of prevention by elim- inating pest food, hiding places, and access. Minimize fish food on the floor or the lid of the tanks through careful feeding methods that result in little spillage, as well as good sanitation in all areas. Control pest access by tightly closing doors and by blocking conduits and other less obvious paths. Eliminate insect hideouts by treating spaces behind walls and between wall studs with boric acid and removing unnecessary paper and cardboard. Sodium borate treat- ment is fairly effective against insects and is unlikely to harm the fish.

Emergency, weekend, and holiday care: An emergency or disaster plan should address all reasonably foreseeable situa- tions. With electrical power outages and mechanical equipment failures high on the list of failure scenarios, minimal precau- tions include having backup equipment and an electrical generator.

Weekend and holiday care should pro- vide at least minimal feeding for adults and feeding and care for the baby fish. Adult fish can tolerate a few days without food but require daily feeding for optimal egg production. Because larval fish require normal care daily, it may be advisable to schedule fewer fish in the nursery during holidays to reduce labor requirements.

Population Management

Identification and records: Identification labels placed on zebrafish tanks should contain information about the genetic background, stock number, the date of fertilization, and the researcher's name. In larger facilities, these records may be kept in computer database files. A downloadable version of such a database can be obtained from the University of Oregon Zebrafish Facility website (http://darkwing.uoregon.edu/-zfish/). Some facilities also use barcodes to automate and reduce data entry error. More sophisticated systems have their files on a dedicated server powered by an uninter- ruptible power source (UPS), with auto- matic backup.

Genetics and nomenclature: Genetic nomenclature guidelines have been estab- lished for zebrafish mutations and wild- type lines. These guidelines are briefly explained on the Zebrafish Information Network (ZFIN) nomenclature page (http://zfish.uoregon.edu/). Zebrafish genetic composition is determined from the records of the parents that generated the fish, phenotypic classifications of the fish and their siblings, and genetic and molecular tests to determine if fish carry particular recessive traits.

Veterinary Medical Care

Animal Procurement and Transportation

Specific zebrafish mutants and wild-type lines can be obtained from stock centers, other laboratories, or commercial dealers. The ZFIN website lists many suppliers.

Stock centers and academic laboratories generally provide the expected mutations and wild-type strains. Their facilities vary somewhat in their health surveillance and quarantine procedures. Some will ship out bleached eggs (facilitating the quarantine procedures of the receiving institution). An academic institution's shipment response time can vary as a result of limited availability of rarely requested mutant strains. In contrast, purchases from commercial dealers are usually genetically poorly defined "wild types."

Zebrafish are frequently transported between laboratory facilities. Transporting fish over the short distances on a campus can be as simple as placing adult fish in a bag in a box or, more commonly, in a small covered plastic box (Tupperware' or poly- carbonate filled with fish system water). Eggs or larvae can be transported in cov- ered petri plates, beakers, or tissue culture flasks.

Transporting zebrafish long distances to other facilities is more complicated. The packing requirements are more stringent. Adult fish should be double-bagged in a good-quality plastic fish shipping bag at a density of about 10 fish to a half gallon of water. The bag should be about two-thirds full of air or oxygen. Food should be withheld for a day before shipping so that fish will produce less ammonia while confined. Amquel (a commercially available amrnonia sequestrator) can be added to bind up any ammonia that is produced. Zebrafish eggs and larvae are usually packed 200-300 to a 250- to 500-mi tissue culture flask filled ~50-90% with water. Ideally, the sender bleaches the eggs and places them in sterile water. Methylene blue (0.5 mg/liter or 0.5 p.p.m.) can also be added to the water to reduce fungal growth. The packing box should be insulated and any extra space filled with packing chips.

For international shipments, additional paperwork may be necessary, including a letter to customs agents, a customs invoice, and perhaps fish import permits. Forms that designate certain customs agents and specific airports can facilitate the shipment handling, as well. Unlike domestic shipments, international shipments are often timed to be in transit during the weekend.

Preventive Medicine

Quarantine, stabilization, and separation: AU newly acquired zebrafish should be quarantined to avoid introducing disease into a colony. The health status of incoming fish is rarely known, and the stress of shipping can often cause underlying diseases to become apparent. The preferred quarantine method is absolute, wherein only surface-sanitized embryos are introduced into a facility. An isolated location and water system should be designated as the quarantine area. This can range from a simple individual aquarium with a self-contained filter unit to an entire separate room with many tanks and a flowthrough water system. Newly arrived fish should be equilibrated over an hour or two by floating the bag in the new tank and periodically introducing a small amount of the new tank water into the bag. if the shipment water is in poor condition, it may be necessary to transfer the fish immediately. Once introduced into their new tank, the quarantined fish remain there for three to four weeks. During this time, the fish are observed closely for signs of disease and treated, if necessary. The new fish are then bred and the embryos are surface-sanitized with a mild bleach solution (35 mg/liter sodium hypochlorite for 5 min). Only these sanitized embryos are introduced into the main aquarium facility. Upon confirmation that the fish strain has been successfully established from these embryos, the adults in the quarantine area are euthanized.

Surveillance, diagnosis, treatment, and control of disease: Fish should be observed daily for signs of illness or injury. Signs of disease in fish include color changes (paleness, redness, hemorrhage), changes in shape (weight loss, bloating, skeletal deformity, masses/swellings, exophthalmia), external lesions (ulcerations, fin erosion, gas bubbles, protruding scales), behavioral alterations (rapid breathing, loss of equilibrium, lethargy, erratic movements, gathering at water surface), and increased mortality. When moribund or dead fish are noted, a clinical investigation is warranted and a veterinarian trained in aquatic animal medicine should be consulted. Water quality and husbandry conditions should be reviewed. Depending on the problem, diagnostic workups can include skin scrapings, fin biopsy, gig biopsy, necropsy, bacteriology, virology, and histopathology. Histopathology is a particularly useful diagnostic technique, because the tiny zebrafish can be fixed and sectioned whole, permitting the examination of all primary organs on a single microscope slide. A use- ful tool for monitoring recirculating water systems is the sentinel fish. Kept in a separate tank that is fed with water from the dirty or return water sump, these fish are sampled periodically for disease investigation (**Fig. 1**). The Zebrafish International Resource Center Pathology Service is one

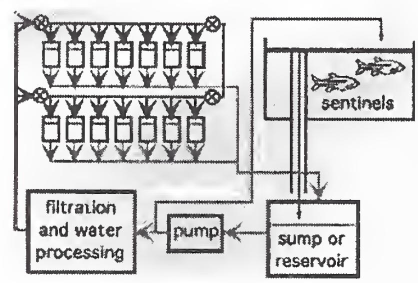

FIGURE 1. Schematic of a sentinel fish tank on a water system. A mixture of potential pathogens are collected from all the tanks served by the water system in the water flowing into the return water sump. This untreated water (red) is then pumped out of the sump to be filtered and UV-irradiated before it is returned to the tanks (black). A side stream (blue) is directed into the sentinel tank before any filtration or irradiation treatment. The sentinel fish thus provide a good, easily sampled system for detecting any pathogens being shed by any of the fish served by the water system.

pathology service that offers complete diagnostic services and consultations on zebrafish health and husbandry. (see http://zfin.org/zf_info/stckctr/health.html)

Most treatments for zebrafish disease have been adapted from the tropical pet fish industry or food fish aquaculture. Among the many considerations involved in initiating a treatment protocol are the diagnosis, the number of fish being treated, the drug and route of administration, and the potential for toxicity to biological filtration. Before starting a generalized treatment, one should always test a drug on a few fish.

Surgery

The most common surgical procedure done on zebrafish is a caudal fin clip to collect tissue for DNA isolation and PCR analysis. The technique permits screening for fish carrying mutations in a particular gene. With practice one can do the proce- dure very rapidly and cause no bleeding. No presurgical cleansing of the caudal fin should be necessary, but gloves should be worn and the surgical area should be clean. Before doing surgery, small (500 ml) individual tanks containing clean fish water for anesthesia recovery and holding should be set up. Individual fish should be identified and isolated until the PCR analysis is complete. Another surgical manipulation of zebrafish is tattooing.

Pain, Anesthesia, and Analgesia

Although it is generally accepted that fish do experience pain, it can be difficult to assess. Signs of pain or distress could include escape behavior or frantic movements, increased respiration (rapid movement of opercula), and blanching of color. To decrease overall stress, fish are anesthetized for all procedures that could cause pain and distress or that require temporary immobilization. The most common anesthetic agent used is MS-222 (tricaine methanesulfonate) in an aqueous solution. Fish are induced rapidly following immersion in a solution containing MS-222 (I 00- 200 mg/L) [12,19] and are recovered by returning them to fresh, well-aerated water.

Because most procedures performed on zebrafish are very rapid, the need for a maintenance phase of anesthesia is usually not necessary. Maintenance anesthesia doses would be lower (50-100 mg/L)19. During induction, spontaneous ventilation should be monitored closely and can be used as an indicator to the depth of anesthesia.

Euthanasia

Zebrafish should be euthanized by methods consistent with the *2000 Report of the AVMA Panel on Euthanasia. The* method chosen depends on the researcher, facility, or the intended use of the fish after euthanasia. An overdose of MS-222 is the most common method, using a slightly more concentrated solution (e.g., 200-500 mg/liter) than is typical for anesthesia. Fish are left in the MS-222 solution for 5-10 min following the cessation of opercular movement. A second method of cuthaniz- ing zebrafish is immobilization by submer- sion in ice water followed by cranial con- cussion and decapitation using an in-sink garbage disposal. Another method, useful when tissues must be preserved, is anesthe- sia with MS-222 followed by quick freezing in liquid nitrogen.

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Guidelines for Use of Fish in Field Research

AFS Policy Statement #16

(Abbreviated)

Policy Statement #16 (Abbreviated). American Fisheries Society (AFS), American Society of Ichthyologists and Herpetologists (ASIH), and American Institute of Fishery Research Biologists (AIFRE) (1987) Guidelines for the Use of Fishes in Field Research. Approved Sept 1987, Winston-Salem, NC.
http://www.fisheries.org/Public_Affairs/Policy_Statements/ps_16a.shtml

Respect for all forms and systems of life is an inherent characteristic of scientists and managers who conduct field research on fishes. The respectful treatment of wild fishes in field research is both an ethical and a scientific necessity. Traumatized animals may exhibit abnormal physiological, behavioral, and ecological responses that defeat the purposes of the investigation. Because of the very considerable range of adaptive diversity represented by the over 20,000 species of fishes, no concise or specific compendium of approved methods for field research is practical or desirable. The ultimate responsibility for the ethical and scientific validity of an investigation and the methods employed must rest with the investigator.

The AFS policy regarding the use of fish in field research includes all phases of handling fish:

1. Collecting

- The number of specimens collected should be kept to the minimum the investigator determines necessary to accomplish study goals.
- Capture techniques should be as environmentally benevolent as possible within the constraints of sampling design.
- Current literature should be reviewed to ascertain when and if capture distress has been properly documented.
- Trap and net sets should be examined at a regular and appropriate schedule, particularly in warm water, to avoid excessive net mortality.

- Use of ichthyocides should be accomplished with maximal consideration of physical factors such as water movement and temperature, so as to avoid extensive mortality of natural populations and nontarget species.
- Electric currents used for electrofishing should be adjusted so as to minimize injury and harm to both the operator and the fish being collected.
- Hooks and spears are an appropriate sampling gear in special environments where other gear cannot be used.
- Fishes collected for museum deposition should be fixed and preserved so as to assure the maximum utility of each animal and to minimize the need for duplicate collecting.
- Fishes that do not die rapidly following immersion in a formalin solution should be killed before preservation by means of a chemical anesthetic such as sodium pentobarbital, hydrous chlorobutanol, MS-222, urethane or similarly acting substances, unless justified in writing by the investigator.
- When field fixation of formalin-resistant fishes without prior introduction of anesthetics is necessary, prior numbing of the specimen in ice water should be considered.
- Live capture should be designed to prevent or minimize injury to the animal.
- Care should be exercised to avoid accidental capture of non-target species. Those captured should be released immediately.
- Collection should be conducted so as to leave the habitat as undisturbed as possible.
- The collection of a large series of animals from breeding aggregations should be avoided if possible.

2. Restraint and Handling

- Investigators must use the least amount of restraint necessary to do the job.
- When not under study, aggressive species should not be confined with other animals (other than food) which they may injure or which may injure them.
- Animals should be handled quietly and with the minimum personnel necessary.
- Darkened conditions which alleviate stress and subdue certain species should be used whenever possible and appropriate.
- When hazardous species such as sharks are handled, (a) chosen procedures should minimize the amount of handling to reduce or eliminate contact between handler and animal, (b) a second person, knowledgeable in capture and handling techniques and emergency measures, should be present at all times, and (c) consultation with experienced workers as well as review of literature is recommended because much information on the handling of hazardous species is passed on from person to person rather than through published literature.
- Prolonged distressful restraint of hazardous fish should be avoided. In some cases, use of a general anesthesia may be advisable.
- Chemicals chosen for immobilization should consider the impacts of the chemical on target organisms.
- Users must be aware of appropriate action necessary in the event of accidental human intake of hazardous drugs used for fish collection and handling.

3. Animal Marking

- Careful testing of markers on preserved or captive animals before use on wild animals is recommended to determine effects on behavior, physiology, and survival.
- Investigators must also consider the nature and duration of restraint, the amount of tissue affected, whether distress is momentary or prolonged, whether the animal, after marking will be at greater than normal risk, whether the animal's desirability as a mate is reduced, and whether the risk of infection or abscess formation is minimal.
- Fin clipping is a recommended procedure, but the importance of each fin to the survival and well-being of target fishes must be determined on a case-by-case basis before being used.
- Marking techniques involving tissue removal or modification (branding, etc.) should be preceded by local anesthetic (aerosols containing benzocaine, such as Cetacaine, may be applied) and followed by the application of a topical antiseptic.
- Tags should not be used which could cause physical impairment or enhance the risk of entanglement in underwater vegetation.
- Brightly colored tags which compromise a fish's camouflage should not be used.
- The size, shape, and placement of tags should permit normal behavior of the animal to the greatest extent possible.
- Force-fed radiotelemetry packages should be small enough to pass through the gut without obstructing the passage of food.
- Force-fed or implanted radiotelemetry packages should be coated with an impervious, biologically inert coating.
- Implanted radio transmitters should not interfere with the function of the organs surrounding them or with the fish's normal behavior.
- Externally attached radio transmitters should neither conceal nor enhance the appearance of dorsal fins or opercular flaps, and should be attached so as to eliminate or minimize the risk of entanglement with underwater vegetation or other obstructions.
- Special training and precautions should be taken before radioisotopes are used for marking fish. A license, which specifies safety procedures for laboratory use, is required for release of isotopes into natural systems and for disposal of waste material.

4. Housing and Maintenance at Field Sites

- Restraint and ease of maintenance by animal keepers should not be the prime determinants of housing conditions.
- Normal field maintenance should incorporate, as far as possible, those aspects of the natural habitat deemed important to the survival and well-being of the animal.
- Nutritionally balanced diets should be provided or natural foods should be duplicated as closely as possible.
- Natural light and temperature conditions should be followed unless alterations of these are factors under investigation.

- Frequency of aquarium cleaning should represent a compromise between the level of cleanliness necessary to prevent disease and the amount of distress imposed by frequent handling and exposure to unfamiliar surroundings.

5. Disposition Following Studies

- Upon completion of studies, researchers should release wild-caught specimens whenever this is practical and ecologically appropriate. Exceptions are if national, state, or locals laws prohibit release, or if release might be detrimental to the well-being of the existing gene pools of native fishes in a specific geographic area.
- Field-captured fishes should be released only: (a) at the site of the original capture, unless conservation efforts or safety considerations dictate otherwise (release should never be made beyond the native range of the distribution of a fish without prior approval of the appropriate state and federal agencies, and approved relocations should be noted in subsequent publication of research results), (b) if their ability to survive in nature has not been irreversibly impaired, (c) where it can be reasonably expected that the released animal will function normally within the population, (d) when local and seasonal conditions are conducive to survival, and (e) when release is not likely to spread pathogens.
- Captured animals that cannot be released or are not native to the site of intended release should be properly disposed of, either by distribution to colleagues for further study or, if possible, by preservation and disposition as teaching or voucher specimens in research collections.
- Investigators must be careful to ensure that animals subjected to a euthanasia procedure are dead before disposal.
- In those rare instances where specimens are unacceptable for deposition as vouchers or teaching purposes, disposal of carcasses must be in accordance with acceptable practices as required by applicable regulations.
- Animals containing toxic substances or drugs (including euthanasia agents like T-61) must not be disposed of in areas where they may become part of the natural food web.

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GUIDELINES FOR USE OF FISHES IN FIELD RESEARCH

American Society of Ichthyologists and Herpetologists (ASIH)
American Fisheries Society (AFS)
American Institute of Fisheries Research Biologists (AIFRB)

from *Fisheries*, Vol. 13, No. 2, p. 16-23, 1988

Introduction

Respect for all forms and systems of life is an inherent characteristic of scientists and managers who conduct field research on fishes. Consistent with our long standing interests in conservation, education, research and the general well-being of fishes, the ASIH, AFS, and AIFRB support the following guidelines and principles for scientists conducting field research on these animals. As professional scientists sp g in fish biology concerned with the welfare of our study animals, we recognize that guidelines for the laboratory care and use of domesticated stocks of fishes are often not applicable to wild-caught fishes, and in fact may be impossible to apply without endangering the well-being of these fishes. Laboratory guidelines may also preclude techniques or types of investigations known to have minimal adverse effects on individuals or populations (1.2.3), and which are necessary for the acquisition of new knowledge.

The respectful treatment of wild fishes in field research is both an ethical and a scientific necessity. Traumatized animals may exhibit abnormal physiological, behavioral and ecological responses that defeat the purposes of the investigation. For example, animals that are captured, marked and released must be able to resume their normal activities in an essentially undisturbed habitat if the purposes of the research are to be fulfilled.

The acquisition of new knowledge and understanding constitutes a major justification for any investigation. All effects of possibly valuable new research procedures (or new applications of established procedures) cannot be anticipated. The description and geographic distribution of newly discovered species justifies studies of organisms that are poorly known. It is impossible to predict all potential observation or collection opportunities at the initiation of most fieldwork, yet the observation or acquisition of unexpected taxa may be of considerable scientific value. Field studies of wild fishes often involve many species, some of which may be unknown to science before the onset of a study. A consequence of these points is that frequently investigators must refer to taxa above the species level as well as to individual species in their research design.

Because of the very considerable range of adaptive diversity represented by the over 20,000 species of fishes, no concise or specific compendium of approved methods for field research is practical or

desirable. Rather, the guidelines presented below build on the most current information to advise the investigator, who will often be an authority on the biology of the species under study, as to techniques that are known to be appropriate and effective in the conduct of field research. Ultimate responsibility for the ethical and scientific validity of an investigation and the methods employed must rest with the investigator. To those who adhere to the principles of careful field research these guidelines will simply be a formal statement of precautions already in place.

General Considerations

Research proposals may require approval of an IACUC (see below). In situations requiring such approval, each investigator must provide written assurance in applications and proposals that field research with fishes will meet the following requirements.

a. The living conditions of animals held in captivity at field sites will be appropriate for fishes and contribute to their health and well-being. The housing, feeding and nonmedical care of the animals will be directed by a scientist (generally the investigator) trained and experienced in the proper care, handling, and use of the fishes being maintained or studied. Some experiments (e.g. competition studies) will require the housing of mixed species in the same enclosure. Mixed housing is also appropriate for holding or displaying certain species.
b. Procedures with animals must avoid or minimize distress to fishes, consistent with sound research design.
c. Procedures that may cause more than momentary or slight distress to the animals should be performed with appropriate sedation, analgesia, or anesthesia, except when justified for scientific reasons in writing by the investigator.
d. Fishes that would otherwise experience severe or chronic distress that cannot be relieved will be euthanized at the end of the procedure or, if appropriate, during the procedure.
e. Methods of euthanasia will be consistent with the rationale behind the recommendations of the American Veterinary Medical Association (AVMA) Panel on Euthanasia (4), but fishes differ sufficiently that their specific techniques do not apply. The method listed by the Royal Society (5) may be followed.
Additional general considerations that should be incorporated into any research design using wild fishes include the following:
f. The investigator must have knowledge of all regulations pertaining to the animals under study, and must obtain all permits necessary for carrying out proposed studies. Investigators must uphold not only the letter but also the spirit of regulations. [Most applicable regulations are referenced in publications of the Association of Systematics Collections (6, 7, 8)] Researchers working outside the United States should ensure that they comply with all wildlife regulations of the country in which the research is being performed. Work with many species is regulated by the provisions of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (see "CITES" references in 6, 7). Regulations affecting a single species may vary with country. Local regulations may also apply.
g. Individuals of endangered or threatened taxa should neither be removed from the wild (except in collaboration with conservation efforts), nor imported or exported except in compliance with applicable regulations.
h. Investigators must be familiar with the fishes to be studied and their response to disturbance, sensitivity to capture and restraint and, if necessary, requirements for captive maintenance to the extent that these factors are known and applicable to a particular study.
i. Taxa chosen should be well-suited to answer the research question(s) posed.
j. Every effort should be made prior to removal of fishes (if any) to understand the population status (abundant, threatened, rare, etc.) of the taxa to be studied, and the numbers of animals removed from the wild must be kept to the minimum the investigator determines is necessary to accomplish the goals of the study. This statement should not be interpreted as proscribing study and/or collection of uncommon species. Indeed, collection for scientific study is crucial to understanding why a species is uncommonly observed.
k. The number of specimens required for an investigation will vary greatly, depending upon the questions being explored. As discussed later in these guidelines, certain kinds of investigations require collection of relatively large numbers of specimens, although the actual percent of any population taken will generally be very small. Studies should use the fewest animals necessary to reliably answer the questions posed. Use of adequate numbers to assure reliability is essential, as studies based on insufficient numbers of fishes will ultimately require repetition, thus wasting any benefit derived from any animal distress necessarily incurred during the study.

Numerous publications exist that will assist investigators and animal care committees in implementing these general guidelines; a number of such journals, monographs, etc. are listed in Appendix A.

Role of the Institutional Animal Care and Use Committee (IACUC)

Field resources for the care and use of fishes are very different from laboratory resources, and the role of the IACUC necessarily is limited to considerations that are practical for implementation at locations where field research is to be conducted. Prevailing conditions may prevent investigators from following these guidelines to the letter at all times. Investigators must, however, make every effort to follow the spirit of these guidelines to every extent possible. The omission from these guidelines of a specific research or husbandry technique must not be interpreted as proscription of the technique.

The IACUC must be aware that while fishes typically used in laboratory research represent a small number of species with well understood husbandry requirements, the classes Agnatha, Chondrichthyes, and Osteichthyes contain at least 20,000 distinct species with very diverse and often poorly known behavioral, physiological and ecological characteristics. Therefore, "... in most cases, it is impossible to generate specific guidelines for groups larger than a few closely related species. Indeed, the premature stipulation of specific guidelines would severely inhibit humane care as well as research." (9). The IACUC must note the frequent use of the word "should" throughout these guidelines, and be aware that this is in deliberate recognition of the diversity of animals and situations covered by the guidelines. Investigators, on the other hand, must be aware that use of the word "should" denotes the ethical obligation to follow these guidelines when realistically possible.

Before approving applications and proposals or proposed significant changes in ongoing activities, the IACUC shall conduct a review of those sections related to the care and use of fishes and determine that the proposed activities are in accord with these guidelines, or that justification for a departure from these guidelines for scientific reasons is presented.

When field studies on wild vertebrates are to be reviewed, the IACUC must include personnel who can provide an understanding of the nature and impact of the proposed field investigation, the housing of the species to be studied, and knowledge concerning the risks associated with maintaining certain species of wild vertebrates in captivity. Each IACUC should therefore include at least one institution-appointed member who is experienced in zoological field investigations. Such personnel may be appointed to the committee on an ad hoc basis to provide necessary expertise. When sufficient personnel with the necessary expertise in this area are not available within an institution, this ad hoc representative may be a qualified member from another institution.

Field research on native fishes usually requires permits from state and/or federal wildlife agencies. These agencies review applications for their scientific merit and their potential impact on native populations, and issue permits that authorize the taking of specified numbers of individuals, the taxa and methods allowed, the period of study, and often other restrictions that are designed to minimize the likelihood that an investigation will have deleterious effects. Permission to conduct field research rests with these agencies by law, and the IACUC should seek to avoid infringement on their authority to control the use of wildlife species.

If manipulation of parameters of the natural environment (day length, etc.) is not part of the research protocol, field housing for fishes being held for an extended period of time should approximate

natural conditions as closely as possible while adhering to appropriate standards of care (10,11). Housing and maintenance should provide for the safety and well-being of the animal, while adequately allowing for the objective of the study.

An increasing body of knowledge (e.g., 12) indicates that pain perception of the many species of vertebrates is not uniform over the various homologous portions of their bodies. Therefore, broad extrapolation of pain perception across taxonomic lines must be avoided. For example, what causes pain and distress to a mammal does not cause an equivalent reaction in a fish (13).

Field Activities with Wild Fishes

1. Collecting

Field research with fishes frequently involves capture of specimens, whether for preservation, data recording, marking, temporary confinement, or relocation. While certain of these activities are treated separately below, they form a continuum of potential field uses of fishes.

The collection of samples for museum preservation from natural populations is critical to: 1) understanding the biology of animals throughout their ranges and over time; 2) the recording of biotic diversity, over time and/or in different habitats; and, 3) the establishment and maintenance of taxonomic reference material essential to understanding the evolution and phylogenetic relationships of fishes and for environmental impact studies. The number of specimens collected should be kept at the minimum the investigator determines necessary to accomplish the goal of a study. Some studies, e.g. diversity over geographic range or delineation of variation of new species, require relatively large samples.

Capture Techniques

Capture techniques should be as environmentally benevolent as possible within the constraints of the sampling design (14, 15). Whenever feasible, the potential for return to the natural environment must be incorporated into the sampling design. Current literature should be reviewed to ascertain when and if capture distress has been properly documented. Those capture techniques (seines, traps, etc.) that have minimal impact on the target fishes are not discussed below. Many capture techniques must mimic those of commercial and recreational fishermen in order to obtain reliable data on population trends for the regulation of such fisheries.

Gill netting (15, 16) and other forms of entangling nets are an accepted practice in fish collecting. Many studies contrast recent and prior sampling and thus repetition of a prior technique is mandated for sampling reliability. Net sets should be examined at a regular and appropriate schedule, particularly in warm water, to avoid excessive net mortality.

Collecting fish using ichthyocides is often the only and by far the most efficient sampling technique (cf. 17). Use of ichthyocides should be accomplished with, maximal consideration of physical factors such as water movement and temperature, so as to avoid extensive mortality of natural populations and non target species.

Electrofishing is a suitable sampling technique in water of appropriate conductivity inasmuch as fish mortalities will be minimal. Proper adjustment of current will stun fishes and complete recovery is possible. Fish can be returned with minimal adverse impact. Care must be exercised to avoid excessive electric currents that may injure or harm the operators as well as the fish.

Capture of fishes by hooks or spears is an accepted practice of recreational fishermen. Spearfishing is appropriate to cases in which capture in special environments is necessary, e.g. deep reefs, caves, kelp beds, etc., and to provide comparable data for recreational fishing statistics. Similarly, many fishes are most efficiently captured by hooks.

Museum Specimens and Other Killed Specimens

The collection of live animals and their preparation as museum specimens is necessary for research and teaching activities in systematic zoology and for many other types of studies. Such collections should further our understanding of these animals in their natural state. Descriptions of ichthylogical collecting techniques and accepted practices of collection management have been compiled (18, 19), as have references to field techniques. Whenever fishes are collected for museum deposition, specimens should be fixed and preserved so as to assure the maximum utility of each animal and to minimize the need for duplicate collecting. In principle, each animal collected should serve as a source of information on many levels of organization from behavior to DNA-sequencing. Whenever practical for example, blood and other tissues should be collected for karyotypic and molecular study prior to formalin fixation of the specimen (20).

Formalin fixation of specimens is an acceptable practice; however, fishes that do not die rapidly following immersion in a formalin solution should be killed before preservation by means of a chemical anesthetic such as sodium pentobarbital, hydrous chlorobutanol, MS-222, urethane or similarly acting substances, unless justified in writing by the investigator. When field fixation of formalin resistant fishes without prior introduction of anesthetics is necessary, prior numbing of the specimen in ice water should be considered. Several kinds of anesthetics and their efficacy have been reviewed in the Investigations in Fish Control series (21). Their use requires little additional time and effort and adds little to the bulk or weight of Collecting equipment Urethane has been shown to be carcinogenic; thus, caution should be observed with its use and field disposal.

Live Capture

Investigators should be familiar with the variety of ichthyological capture techniques and should choose a method suited to both the species and the study. Capture techniques should prevent or minimize injury to the animal. Care should be exercised to avoid accidental capture or insure field release of non-target species. The interval between visits to traps and net sets should be as short as possible, although it may vary with species, weather, objectives of the study, and the type of trap or net.

Habitat and Population Considerations

Whether collecting for future release or for museum preparation, each investigator should observe and pass on to students a strict ethic of habitat conservation. Collecting always should be conducted so as to leave the habitat as undisturbed as possible. The collection of large series of animals from breeding aggregations should be avoided if possible. Systematists should be familiar with extant collections of suitable specimens before conducting field work. If the purpose of an experiment is to alter behavior, reproductive potential, or survivability, the interference should be no more than that determined by the investigator to accurately test the hypothesis.

2. Restraint and Handling

General Principles

Restraint of wild fishes ranges from confinement in an aquarium through various types of physical restrictions or drug-induced immobilization. The decision whether to use physical or chemical restraint should be based upon the design of the experiment, knowledge of behavior of the animals, and the availability of facilities. Investigators must use the least amount of restraint necessary to do the job. When not under study aggressive species should not be confined with other animals (other than food) which they may injure or may injure them. The well-being of the animal under study is of paramount importance, and we emphasize that improper restraint, especially of traumatized animals, can lead to major physiological disturbances that can result in any of a series of deleterious or even fatal consequences.

Animals should be handled quietly and with the minimum personnel necessary. Darkened conditions tend to alleviate stress and subdue certain species, and are recommended whenever possible and appropriate.

Hazardous species

Sharks and other large or venomous fishes are potentially dangerous to the investigator, and thus require special methods of restraint that must involve a compromise between potential injury to the handlers and injurious restraint of the animal. The particular method chosen win vary with the species and purpose of the project Adherence to the following general guidelines is recommended when working with hazardous fishes:

a. Procedures chosen should minimize the amount of handling time required and reduce or eliminate contact between handler and animal. b. One should never work alone. A second person, knowledgeable in capture and handling techniques and emergency measures, should be present at all times. c. Prior consultation with workers experienced with these species, as well as a review of the relevant literature, is of particular importance since much of the information on handling dangerous species has not been published, but is simply passed from one investigator to another.

Prolonged distressful restraint should be avoided. In some cases, utilization of general anesthesia for restraint in the field may be advisable. If so, the anesthetic chosen should be a low risk compound that permits rapid return to normal physiological and behavioral status, and the animal must be kept under observation until appropriate recovery occurs. The relatively unpredictable response of some poikilotherms to immobilants or anesthetics under field conditions may contraindicate field use of these chemicals under certain conditions.

Chemical Restraint

Many chemicals used for restraint or immobilization of fishes are controlled by the Federal Bureau of Narcotics and Dangerous Drugs/Drug Enforcement Administration (DEA). A DEA permit is required for purchase or use of these chemicals. Extensive information on these substances and their use is available (22,23), and permit application procedures are available from regional DEA offices. Investigators should choose the chemical for immobilization with consideration of the impacts of that chemical on the target organism.

The potent drugs available for wildlife immobilization when properly used are, with the exception of succinylcholine, safe for target animals but can be extremely dangerous if accidentally administered to humans. The degree of danger varies according to the drug, and users must be aware of the appropriate action to take in the event of accident (9).

3. Animal Marking

Fish marking, by a variety of techniques, provides one of the most important methods of analyzing fish movements, abundance, and population dynamics (cf. 24). It is basic to all field studies. Important considerations in choosing a marking technique are its effect on behavior, physiology and survival of the target species or a close relative. Investigators must consider the nature and duration of restraint, the amount of tissue affected, whether distress is momentary or prolonged, whether the animal, after marking, will be at greater than normal risk, whether the animal's desirability as a mate is reduced, and whether the risk of infection or abscess formation is minimal. Careful testing of markers on preserved or captive animals before use on wild animals may reveal potential problems and is recommended. Marking techniques for fishes have been extensively reviewed (25) and are summarized below.

Fin-clipping is relatively easy, may have minimal impact on survival and social structure of the marked fish, and is a recommended procedure for many studies. Fins used for clipping or removal would depend upon the species selected, i.e., clipping of the anal fin of poeciuid males would be inappropriate, but removal of the adipose fin of a salmonid would have negligible impact. The importance of fins to the survival and well being of fishes varies so widely that specific guidelines are not possible.

Marking techniques involving tissue removal or modification (branding, etc.) should be preceded by local anesthetic (aerosols containing benzocaine, such as Cetacaine, may be applied) and followed by the application of topical antiseptic. Chilling of fishes prior to marking may be effective for immobilization.

Electrocauterization of a number, letter, or pattern on the skin, in which deep layers of skin are cauterized to prevent regeneration, provides a marking system that, if performed properly, heals rapidly and seldom becomes infected. Brand marks typically, however, are not visible in captive fishes after a few months. Freeze branding is often the preferred branding technique.

Tattooing and acrylic paint injections have been used with success on fishes. Two potential problems that must be resolved prior to marking are: 1) the selection of a dye which will be visible against the pigmentation of the skin, and 2) the loss of legibility due to diffusion or ultraviolet degradation of the dye.

Tagging is perhaps the most widely used and best investigated means of fish marking. Several logical constraints should be considered in planning any tagging program. Tags that cause projections from the body could produce physical impairment and enhance the risk of entanglement in underwater vegetation. Brightly colored tags may compromise a fish's camouflage. The size, shape and placement of tags should permit normal behavior of the animal to the greatest extent possible.

Radiotelemetry

Radiotelemetry is a special form of animal marking, and the same general procedures apply. Underwater telemetry, however, is primarily limited to acoustic rather than radio frequency transmission. Radio transmission is only practical in freshwater and at relatively shallow depths. Radio transmission is regulated by the Federal Communications Commission, and investigators should inquire about availability of frequencies they plan to use. General telemetry techniques are summarized by Mackay (26), Amlaner and MacDonald (27), and Stasko and Pincock (28).

Many fishes are unsuitable for radiotelemetric studies because of their small size and habit of living in confined spaces. Component miniaturization will undoubtedly facilitate the future use of radiotelemetry in studies of small fish species, particularly with internally implanted transmitters.

Researchers intending to use radiotelemetry on fish species should consider the following guidelines and comments:

a. *Force-fed and Implanted Transmitters*: Force-fed packages should be small enough to pass through the gut without obstructing the passage of food. Force-fed or implanted packages should be coated with an impervious, biologically inert coating. Residence time of up to several days in the gut is generally long enough to provide useful information on movement and body temperature. Implanted transmitters should not interfere with the function of the organs surrounding them or with the fish's normal behavior. For intracoelomic or subcutaneous implants, the transmitter package may have to be sutured in place to prevent its movement or interference with vital organs.
b. *Externally Attached Transmitters*: Consideration must be given to the effect of an externally attached transmitter package on behavioral interactions between tagged fishes and other individuals. For example, the transmitter should neither conceal nor enhance the appearance of dorsal fins or opercular flaps. Transmitters should be shaped and attached so as to eliminate or minimize the risk of entanglement with underwater vegetation or other obstructions.

Most fishes continue to grow throughout life. External transmitters should be removed or designed to be lost after a time, or they may constrict or irritate the animals. Special consideration must be given to soft-skinned species to prevent abrasion.

Radioisotopes

The use of radioisotopes as markers in natural systems is very valuable, and may be the only means of adequately gathering data on movements of very small species; the technique, however, should be undertaken with caution. Special training and precautions are required of researchers by federal, and frequently state law (8). A license, which specifies safety procedures for laboratory use, is required for release of isotopes into natural systems and for disposal of waste material. The pros and cons of using strong emitters must be assessed in terms of possible deleterious effects on the animal, to predators that might ingest isotope-labeled animals, and potential hazard to the public.

When marking with radioisotopes, the animal does not have to be handled for identification, several individuals can be monitored rather quickly, the label is easy to apply, and it can be useful for a limited time if desired. Strong emitters, however, cause extensive tissue necrosis at the implant site, and even weaker ones carry the chance for induction of mutations that may compromise future genetic studies of these populations.

Housing and Maintenance at Field Sites

Because the biological needs of each species and the nature of individual projects vary widely, only the most general recommendations on housing wild vertebrates in the field can be made. When dealing with unfamiliar species, testing and comparing several methods of housing to find the method most appropriate for the needs of the animal and the purposes of the study may be necessary. *Restraint and ease of maintenance by animal keepers should not be the prime determinants of housing conditions, though these are certainly important considerations.*

Normal field maintenance should incorporate, as far as possible, those aspects of the natural habitat deemed important to the survival and well-being of the animal. Adequacy of maintenance can be judged, relative to the natural environment, by monitoring a combination of factors such as changes in growth and weight, survival rates, breeding success, activity levels, general behavior, and appearance (29). Nutritionally balanced diets should be provided or natural foods should be duplicated as closely as possible. Natural light and temperature conditions should be followed unless alterations of these are factors under investigation.

Frequency of aquarium cleaning should represent a compromise between the level of cleanliness necessary to prevent disease (30,31,32), and the amount of distress imposed by frequent handling and exposure to unfamiliar surroundings. Applied knowledge of animal ethology can assist the investigator in providing optimum core and housing.

Disposition Following Studies

Upon completion of studies, researchers should release wild-caught specimens whenever this is practical and ecologically appropriate. Exceptions are: if national, state, or local laws prohibit release, or if release might be detrimental to the well-being of the existing gene pools of native fishes in a specific geographic area.

As a general rule, field captured fishes should be released only:

a. At the site of the original capture, unless conservation efforts or safety considerations dictate otherwise. Release should never be made beyond the native range of distribution of a fish without prior approval of the appropriate state and/or federal agencies, and approved relocations should be noted in subsequent publication of research results.
b. If their ability to survive in nature has not been irreversibly impaired.
c. Where it can be reasonably expected that the released animal will function normally within the population.
d. When local and seasonal conditions are conducive to survival.
e. When release is not likely to spread pathogens.

Captured animals that cannot be released or are not native to the site of intended release should be properly disposed of, either by distribution to colleagues for further study, or if possible by preservation and deposition as teaching or voucher specimens in research collections.

In both the field and laboratory, the investigator must be careful to ensure that animals subjected to a euthanasia procedure are dead before disposal. In those rare instances where specimens are unacceptable for deposition as vouchers or teaching purposes, disposal of carcasses must be in accordance with acceptable practices as required by applicable regulations. Animals containing toxic

substances or drugs (including euthanasia agents like T-61) must not be disposed of in areas where they may become part of the natural food web.

Preparation and Revisions of these Guidelines

The initial draft of these guidelines was prepared by Clark Hubs (ASIH), John G. Nickel (AFS) and John R. Hunter (AIFRB). The final product represents the collective efforts of over 100 persons and the societies extend sincere thanks to all participants.

Periodic revision of these guidelines is expected. Investigators are encouraged to send constructive criticisms of applicable new information to officers of the societies.

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2.11.

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Chapter 7 Fish Welfare and Health in Relation to Tagging, written by Prof. John Davenport (group leader), Dr. Etienne Baras, Dr. Gianna Fabi, and Dr. Gisli Jonsson, excerpted from **Thorsteinsson V (2002) Tagging Methods for Stock Assessment and Research in Fisheries, Report of Concerted Action FAIR CT.96.1394 (CATAG). Reykjavik. Marine Research Institute Technical Report (79), pp 179**, is reprinted in full with permission from V. Thorsteinsson (Coordinator of CATAG), Chapter 7 Authors listed above, and members of CATAG. The entire CATAG report document is available online at http://www.hafro.is/Timarit/catag.pdf. For more information about CATAG, please see their website at http://www.hafro.is/catag/.

7. FISH WELFARE AND HEALTH IN RELATION TO TAGGING

7.1. INTRODUCTION

All forms of fish tagging involve invasive procedures, first by capture itself. Externally-fixed, or superficially-injected tags breach the skin and musculature, while internal tags (whether mounted in the stomach or peritoneal cavity) normally involve either force feeding or surgery (though some tags can be ingested voluntarily in food/bait). Use of anaesthesia may itself alter body biochemistry (e.g. MS222 use causes elevated serum cortisol levels in coho salmon; Strange & Schreck, 1978). All types of tags have the potential to cause health problems for fish subsequent to the tagging process itself. There may be disturbances of physiological function, or more subtle behavioural or immunological effects.

7.2 ANAESTHESIA

7.2.1. Introduction

Rendering fish quiet (sedation) or unconscious (anaesthesia) is crucial to several aspects of fish tagging. Summary sheets at the end of this section are intended to help operators choose and use anaesthetics: they are also readily downloadable as OHP slides. More information about anaesthesia may also be gained by interrogating the WELFARE database. Operators should be aware that there are legislative implications of use of anaesthetics on fish that are to be released to the wild because of the perceived risk of chemical residues reaching humans through the food chain (see section 6.2.4 in legislation section).

7.2.2. Anaesthesia

A variety of handling methods have been applied during the tagging process, ranging from use of blindfolding in calming fish, to full anaesthesia involving continuous irrigation of the gills with fresh or seawater containing diluted anaesthetic agents.

Under anaesthesia, handling stress will be reduced and tagging can be accomplished more rapidly without risk of the fish hurting themselves when trying to escape. Although the use of anaesthetics in some cases may be unwanted due to their detrimental effects on the physiology and behaviour of the fish, considerations of animal welfare will in most cases prohibit tag attachment to unsedated fish if surgery is involved.

7.2.3. Choice of anaesthetics

Different handling procedures demand different anaesthetic approaches. Light anaesthesia (=sedation) is defined as 'reduced activity and reactions to external stimuli', and is sufficient for procedures such as transport or weighing of fish. Full anaesthesia can be defined as 'loss of consciousness and reduced sensing of pain, loss of muscular tonus and reflexes' and is needed when surgical procedures are applied (MacFarland 1959).

The behavioural changes occurring in fish passing through sedation to full anaesthesia were classified by MacFarland (1959). There are 4 stages with subclasses ranging from normal (stage 0), where the fish reacts to external stimuli and where the muscular tonus and swimming ability is normal, to the stage of total physiological collapse (stage IV), where gill movements have stopped and which in a few minutes will lead to heart failure. In a tagging context, the stages where the fish is in a state of light/deep anaesthesia (stages II and III) are of greatest relevance, as the animal is then insensitive to pain caused by the attachment of transmitters or data storage tags.

Choice of sedatives/anaesthetics must be based on the species to be tagged, the number and size of fish involved, and the duration of the operation in question. Water temperature and chemistry have also to be taken into consideration when choosing the method. Lastly, the work often has to be done under primitive field conditions without accurate control of concentrations and exposure times. An anaesthetic with a good safety margin between effective anaesthesia and irrevocable collapse is essential in such circumstances.

7.2.4. Categories of methods

(a) Physical sedation methods

Physical sedation can be obtained by rapid lowering of temperature or by electric shock. The former method is mainly applicable for transportation (c.f. Ho & Vanstone, 1961). Coldwater adapted species, and marine fish require lower temperatures for sedation than warm water species and freshwater fish (Chung 1980). Water cooling can also be used in conjunction with other anaesthetics (e.g. Benzocaine) but the dosage must then be reduced by about 30% (cf. Ross and Ross 1983). Electroanaesthesia has a number of advantages such as rapid immobilisation of fish, no need for chemicals, rapid regain of consciousness and low costs (Madden and Houston 1976, Gunstrom and Bethers 1985, Tytler and Hawkins 1981; Cowx & Lamarque, 1990; Cowx, 1990). But these are outweighed by the fact that the method cannot be used in saline water, and the danger of using inappropriate voltage levels, which may give severe physiological stress responses in experimental

fish (Shreck *et al.* 1976) due to hypoxia. There are also significant risks to experimenters, principally from electric shock. In the U.K. the National Rivers (NRA) issued a safety Code of Practice in 1995.

(b) Chemical sedation and anaesthesia

Chemical sedation is distributed to fish in liquid dilutions of varying strengths depending on the agent used. The sedative is inhaled by the fish and diffuses across the gill epithelia. In minor quantities it can also diffuse into the fish via the skin (Fereira *et al.* 1984) - this may be a particularly significant route in scaleless fish with well-vascularised skins. Since these chemicals are absorbed and excreted predominantly via the gills, fish with a large surface of gill epithelium for a given body weight (e.g. salmonids) require lower doses of anaesthetics than fish (e.g. eels) with relatively smaller epithelial surfaces (Ross & Ross 1983). Other factors affecting the absorption and excretion of chemicals are the relationship between the surface of the gill epithelium and the body volume, thickness of epithelium, type of anaesthetic, dosage and temperature.

All known anaesthetics have unwanted side effects. Most of them are barbiturates, which lead to unconsciousness, inhibition of the sensing of pain and loss of muscular tonus and reflexes. The most important complication connected with all forms of chemical anaesthesia is hypoxia due to reduced respiration and vascular activity. This leads to physiological changes in the blood (e.g. lowered pH), hypotonia (= reduced blood pressure), raised blood glucose, blood lactate and haematocrit (Tytler & Hawkins 1981). In addition to physiological deterioration of blood parameters, hypoxia can cause brain damage, which interferes with directional orientation (Taylor 1988), or alters temperature preferences (Goddard *et al.* 1974).

Widely used anaesthetics of the barbiturate group are:

MS 222- Tricaine methane sulphonate

Chemical name: ethyl- amino- benzoatemethanesulphonate. MS 222 is probably the most widely used fish anaesthetic world-wide, and there are numerous studies on the physiological effects of this agent (e.g. review by Bell 1987). It is a crystalline powder easily dissolved in fresh and seawater. The recommended dosage for anaesthesia is 50- 100 mg/ l (Klonz 1964; Fereira et al. 1979). It should be observed that MS222 becomes toxic in seawater exposed to sun (Bell 1987). MS222 gives an acid solution and a dosage of 75 mg l^{-1} can cause the pH to fall to 4.0 in soft water (Wedemeyer 1970). This effect can, however, be mediated by adding 5- 6 ml saturated (10%) solution of $NaHCO_3$ to 1 litre of 100 mg l^{-1} solution of MS222.

Benzocaine

Chemical name: Ethyl-p-aminobenzoate. This chemical is also very widely used in fish anaesthesia. It is chemically close to MS- 222, both being derivatives of p- aminobenzoic acid. Benzocaine is a white crystalline powder, which is insoluble in water and has to be dissolved in ethanol in a 'master solution' of 1 g l^{-1} 96% alcohol. The master solution should be stored in a dark bottle, and has a life of up to a year. The recommended dosage is 2.5 ml of this master solution to 10 l of aerated water. With this dosage the animals should be immobilised in 2 - 5 min. and the recovery time will be 5 - 15 min. Benzocaine gives a neutral solution (Egidius 1973). The time to obtain anaesthesia was observed to take 1.5 min longer time for trout (*Salmo trutta*) and 3 min longer for pike (*Esox lucius*) in 7° C water than at 12 ° C (Dawson & Gilderhus 1979). According to Wedemeyer (1970) a comparison between Benzocaine and MS-222 as anaesthetics for salmonids was slightly in favour of

Benzocaine as less metabolic change was observed. More recent studies by Soivio et al. (1977) showed few differences between the two; both caused hyperglycaemia. However, benzocaine caused somewhat lesser hyperglycaemia than MS- 222. With the exception of occasional allergic reactions, health hazards to humans are not normally recorded with the use of benzocaine (MND 1986).

Chlorbutanol- Chlorbutol- Chorethone- Acetochloroform

Chemical name: Chlorbutanol. Although classified as a safe anaesthetic for fish (Johansson 1978), it has not been widely used outside Scandinavia due to health hazards to humans connected with its use. Inhalation of larger quantities may cause unconsciousness, it can also irritate human skin and eyes. Chlorbutanol (Cb) is a crystalline colourless powder that has to be dissolved in ethanol. The usual base solution is 30 g to 100 ml 96% ethanol, and the dose 10 ml base- solution to 10 litres aerated water. Johansson (1978) states that the time for falling into stupor and wakening is inversely dependent to the water temperature, the higher the temperature the lesser the time needed for sedation. The dosage varies somewhat with the size and species of fish but is considered sufficient when the fish rolls on it side after 3-5 min. Chlorbutanol gives a light anaesthesia, but it is normally sufficient when the fish only needs to be handled for a short time handling, such as in tagging (Johansson 1978, Horsberg and Høy 1989). Chlorbutanol is considered a safe anaesthetic for fish, although a study by Hansen and Jonsson 1988 showed an 87 % reduction in return rates of Atlantic salmon (*Salmo salar*) smolts anaesthetised before release in comparison with untreated fish. Chlorbutanol has also been tested on Atlantic halibut (*Hippoglossus hippoglossus*), but with a dosage of 50 ml base solution dissolved in 10 l water. The smallest fish are most rapidly sedated; they also have the shortest recovery time.

Methomidate chloride

Methomidate is a hypnotic (sleeping-agent) and not a barbiturate. It therefore causes less depression of respiration than MS-222 or Benzocaine. This may lead to fewer and less serious side-effects. Methomidate is water-soluble. Mattson & Riple (1989) report an effective concentration of 5 mg l^{-1}. Methomidate was tested on rainbow trout in the early 1980s by Gilderhus & Marking (1987), and showed in these tests to give a relatively long wake-up time and also some mortality after treatment. However, during the late 1980s this anaesthetic has been tested with good results for handling salmonids and other fish in culture, such as cod and halibut at the Department of Aquaculture, Institute of Marine Research, Norway, (Mattson & Riple 1989; Huse, pers. Com.; Furevik, pers. com). From 1992 onwards methomidate has been the only anaesthetic used at the Dept. of Aquaculture (Holme, pers. com.); the only negative feature is the high cost of the product.

Quinaldine

Quinaldine is not easily soluble in water, and is also reported to be irritating to human skin and mucus membranes. Quinaldine-sulphate does not have these negative effects, but gives an acid solution, and must therefore be buffered with sodium bicarbonate (Blasiola 1977). It has been used in acetone solution for the capture of intertidal fish living in rock pools. Reports that it may be carcinogenic currently restrict use.

Propanidide

In a 5% solution this chemical is water-soluble. *Propanidide* seems to have few physiological side effects, and can be used both for short- and long-duration anaesthesia. The main reported asset of

this anaesthetic is that it does not reduce the ventilatory rate of the fish (Ross & Ross 1987). The blood-circulation can also remain unaffected as reported by Veenstra et al. (1987) from studies of *S. fontinalis* embryos and 7 days old alevins of amargosa pupfish (*Cyprinodon nevadensis amargosae*). It has also been tested on carp (Jeney *et al*. 1986) rainbow trout and smolts of Atlantic salmon and sea trout (Siwicki 1984) with good results.

Clove oil

Chemical name: eugenol (4-allyl-2-methoxy-phenol). Recent experiments (Anderson et al. 1997) have shown that clove oil is just as effective an anaesthetic for both juvenile and adult rainbow trout (*Onchorhynchus mykiss*) as MS-222. Clove oil does not affect swimming performance and it also provides swift induction and recovery from anaesthesia. It is regarded as a GRAS ('generally recognised as safe') substance by the US Federal Drugs Administration (FDA) and is suitable for use in field studies where immediate release of the fish into the food chain is required. Anderson *et al.* (1997) have shown that concentrations of 20-40 and 100-120 mg/l will induce light and heavy anaesthesia, respectively. At a concentration of 120 mg/l induction times are significantly faster than MS-222 for both juveniles and adults. At a concentration of 40 mg/l there is no difference for juveniles but induction times are significantly faster for adults. Recovery times for adult fish are rather longer than MS-222 at the higher concentration but no different at the lower concentration.

7.2.5. Information sheets (http://www.hafro.is/catag/f-health&welfare/studies-res_2.htm)

Downloadable information sheets that will assist in the choice of anaesthetics for specific purposes have been prepared; they are displayed in Appendix II (7.10) of this chapter and are also available on the CATAG web site (http://www.hafro.is/catag).

7.3. EFFECTS OF CONVENTIONAL TAGS ON FISH

Consideration of conventional tagging (including procedures such as fin-clipping) will be given here. Generally such tagging procedures are innocuous and there is little or no stress to fish beyond that involved in capture and handling (e.g. chinook salmon, *Onchorhynchus tshawytscha*, Sharpe *et al*., 1998; see also Gjerde & Reftstie, 1988, Hansen, 1988). The main problem associated with tags is that of pathological lesions caused by tagging or fin clipping (Roberts *et al*., 1973a, b, c; Morgan & Roberts, 1976), or indeed any breach of fish skin. Such lesions may be subject to secondary infections and are likely to cause effects on growth rate and reproductive performance. Uncontrolled infections may well be a source of mortality, but it seems probable that this is very rare.

Adipose fin clipping (commonly performed on Pacific salmon) may be detrimental because there is some evidence that these fins are secondary sexual characters, which perform an important function in mate selection.

Most tagging experiments are based on the assumption that the behaviour, growth and survival of tagged fish is similar to that in untagged fish and that data generated from these studies is unaffected by the type of tag used or the tagging procedure implemented. Few studies have been carried out to assess the impact of simple external tags on the behaviour of fish (e.g. Lewis & Muntz, 1984; McFarlane & Beamish, 1990), probably because they are difficult to design and carry out. Furthermore, tag effects are sometimes examined under controlled laboratory experiments, which often provide conditions different from the natural environment.

While many of the internal tags or marks may have minimal or negligible effect on the behaviour of marked fishes (Buckley & Blankenship, 1990), external tags may affect the behaviour of tagged fish. Small individuals may have problems with relatively large tags and the application of the tag may cause problems, such as wounds around the attachment. External tags may affect feeding or evasive behaviour and the fish may therefore be more vulnerable to predation. Especially in demersal fish, tags may become overgrown with algae and/or mussels, becoming heavier and more cumbersome. An external tag that has not been anchored firmly into the muscle may continue to irritate the fish, preventing the wound from healing causing a chronic wound.

Growth of sablefish, *Anoplopoma fimbria*, was found to be affected by the tag or tagging procedure in a comparison of wild, tagged fish with untagged fish (McFarlane & Beamish, 1990). Thus, extrapolating growth information from tagged fish resulted in altered estimates for mortality and mean age at maturity for this species. On the other hand, no effect on growth was observed in similar studies with Arctic char (*Salvelinus alpinus*) (Berg & Berg 1990).

Carlin tagging and fin clippings are commonly used in studies on salmon or trout migration, survival or growth. Saunders & Allen (1967) showed negative effects of this tagging method on survival of Atlantic salmon, *Salmo salar*, implying that mortality estimated from tagged salmon smolts would result in an underestimation of the survival rates to adults. This was confirmed in later studies on the same species by Isaksson & Bergman (1978) and Hansen (1988). The increased mortality was attributed to handling, anaesthesia and marking of fish. Carlin tagging was found to have a higher impact on survival than fin clipping, although the latter was not without impact, probably due to stress from handling and anaesthesia. In a laboratory study on snapper (*Pagrus auratus*), no effect of dart tags on survival or growth was observed on three length sizes of fish during a one-year period (Quartararo & Kearney 1996).

All tagging or marking of fish involves treatment, which disturbs the fish and may stress or harm the fish. Careful handling procedures throughout the capture and marking process are of highest importance. Physiological research has shown fish to be stressed for a prolonged period after handling; for example, levels of lactic acid may be elevated for more than 24 hours after stressing the fish at certain temperatures (Wendt 1965, 1967; Wendt & Saunders, 1973). Histopathological studies on the effects of Disc-dangler tags on Atlantic salmon (Morgan & Roberts, 1976) revealed that external tags of these types can leave severe traumatic wounds which may lead to secondary infection. Similar observations were made by Vogelbein & Overstreet (1987), who reported histopathological problems with internal anchor tags used on spot, *Leiostomus xanthurus*. The incomplete healing of the integument during the life of the fish may affect the normal behaviour of the fish and result in biased estimates of biological parameters.

A possible (and virtually unstudied) effect of all types of external tagging (whether conventional or with electronic tags) is that tags may become fouled, causing enhanced drag, so disadvantaging the fish. Anecdotal evidence has been collected during CATAG of the existence of such fouling (e.g. by barnacles and seaweed) but more investigation is needed. In particular, it would be desirable if systematic fouling trials could be conducted on tags and tag materials - it is quite possible that fouling could be a source of unremarked mortality of tagged fish.

7.4. EFFECTS OF ELECTRONIC TAGS ON FISH

7.4.1. Introduction

Electronic tags have become commonly used during the last decade to monitor movements, activity, physiological responses and reaction to a number of environmental variables in many fish species. The area of electronic tags is in rapid development, and these tags are used by an increasing number of teams and researchers, in an increasing number of species, most of which have never been tagged before. Implicit in these studies is the usual assumption that the tag and the tagging procedures have no significant effect on the data collected. Whereas some authors found no difference between tagged and untagged fish in terms of behaviour, growth or physiology (e.g. Hinch *et al.,* 1996), other studies have documented adverse effects that are dealt with here. Furthermore, only a very small proportion of tagging studies have investigated the actual adverse effects of tagging, and effects on behaviour or physiology have been investigated far less frequently than direct, 'obvious' effects on survival, anatomy or pathology (see Figure 7.1).

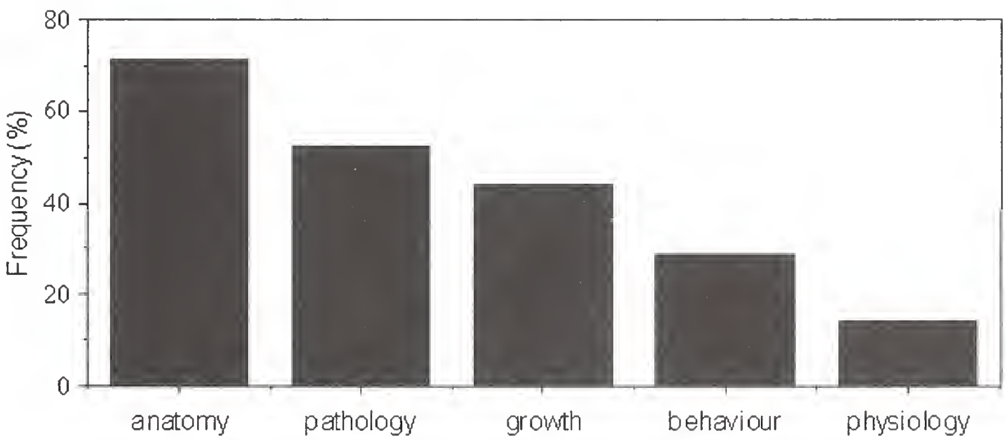

Figure 7.1. Proportion of tagging feasibility studies where the effects of tags or tagging procedure on anatomy, pathology, growth, behaviour and physiology were investigated.

A future goal should be to ensure that the effect of the tag and the tagging procedures on the animals used in any project are studied before this type of assumption can be made with confidence. Furthermore, because electronic tags and tagging techniques are developing rapidly, the need to document modifications of behaviour from newly developed techniques needs to be emphasised. This should be done, not only to secure the welfare of the animals, but also to avoid biased data collection due to decreased performance, altered behaviour or elevated stress level in the fish.

The present review focuses on the effects on fish of tagging and carrying electronic tags. Because of their larger size and mass, telemetry (radio and acoustic) and data storage tags (DST or archival tags) are considered separately from other electronic tags, such as passive integrated transponder (PIT) tags, and from conventional tags (see Section 7.3). The main results from studies dealing with the effects of radio and ultrasonic transmitters in fish are summarised in Appendix I (7.9) of this chapter. Additional, more detailed information can be found in the WELFARE database on the CATAG web site (http://www.hafro.is/catag).

7.4.2. Survival

For ethical considerations, cost effective research and reliable statistical analyses, it is crucial that fish survive the tagging procedure and that neither the tag nor the tagging procedure influence the survival rate of the fish, either during the time of the study or later. Survival rates evaluated in telemetry or DST tagging studies ranged from 20 % one month after tagging (grass carp *Ctenopharyngodon idella*, Schramm & Black, 1984) to 100 % 30 months after tagging (blue tilapia *Oreochromis aurens*, Thoreau & Baras, 1997). Because of differences between the procedures used by different authors (e.g. threads for attachment, coating, tag size, anaesthetics, temperature) and because not all factors likely to influence mortality are systematically investigated, or mentioned in feasibility or field studies, it may be difficult to draw general trends. Different fish species or life history stages may also have different resistances to handling or pathological outbreaks. However, the analysis of the CATAG fish WELFARE data base provides evidence that gastrically-inserted transmitters are less prone to cause the death of fish, compared with externally- attached or intraperitoneally-inserted transmitters (Figure 7.2). Surgical procedures are often deemed to be the most invasive ones, since they require deep anaesthesia, longer handling, opening of the body cavity, and insertion of a foreign body inside the fish. But carefully evaluated procedures tailored to the species of interest are frequently reported to cause no additional mortality compared with controls.

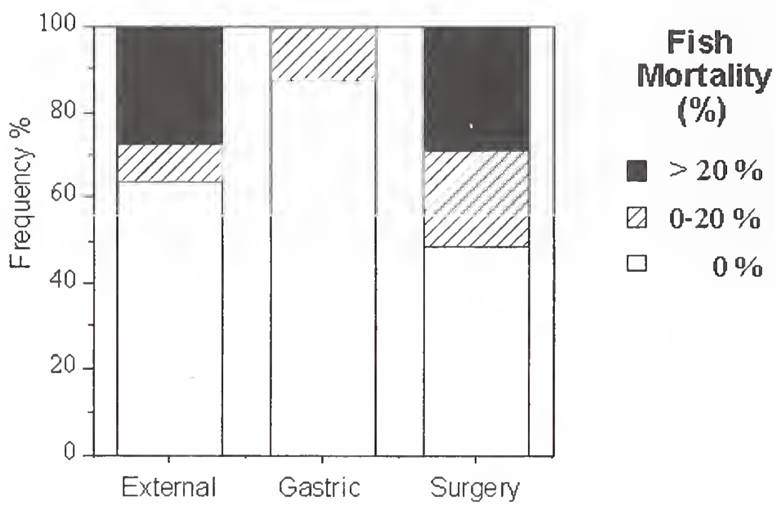

Figure 7.2. Proportion of telemetry studies reporting variable rates (0 %, < 20 %, 20 %) of fish mortality depending on attachment procedure.

Mortality of internally-tagged fish takes place most frequently within the hours, days or weeks following tagging, as a result of wound infection, blockage of gut transit or damage to internal organs. Wood *et al.* (1983) reported that 40 % of tagged rainbow trout *Onchorhynchus mykiss* died within 12 hours following 6 min of intensive exercise, probably because of acidosis. Similarly, most cases of mortality of surgically-implanted fish took place before the fish had healed their incisions and recovered physical integrity and osmotic balance (within 4 days to 7 weeks, depending on species and ambient temperature). In contrast, deaths of externally-tagged fish rarely take place within the first days or weeks. External tag attachment involves progressive, or chronic lesions to muscular tissues, in which degenerative processes exceed by far the capacity for tissue repair (Roberts *et al.*, 1973; Brittles, 1995; Knights & Laze, 1996). Adverse effects thus accumulate over

time and can be exacerbated by exposure to increased water velocity, which increases the drag on the tag. These problems can, however, be postponed depending on the time interval between the moment of tagging and the time of the year when the fish moves into a faster flowing environment. Externally-attached tags or trailing antennas may become entangled in vegetation (e.g. Chinook salmon, *Oncorhynchus tshawytscha* Adams *et al.*, 1998). This can cause tag shedding or fish mortality.

7.4.3. Retention

Tag shedding or expulsion has been reported for all three major attachment procedures (externally-attached, intragastrically-inserted, intraperitoneally-inserted), as well as for oviduct insertion, which has recently been evaluated in salmonids (Peake *et al.*, 1997). Generally, shedding has been reported more frequently, and shedding rates found to be higher for gastrically-inserted tags than for external or intraperitoneal tags (Figure 7.3), and this contrasts with the mortality rates inherent in these three procedures. This section will concentrate on shedding or expulsion mechanisms, and conditions that increase the propensity of fish to shed tags. Details on tag shedding rates in different species or life stages can be found in the WELFARE data base.

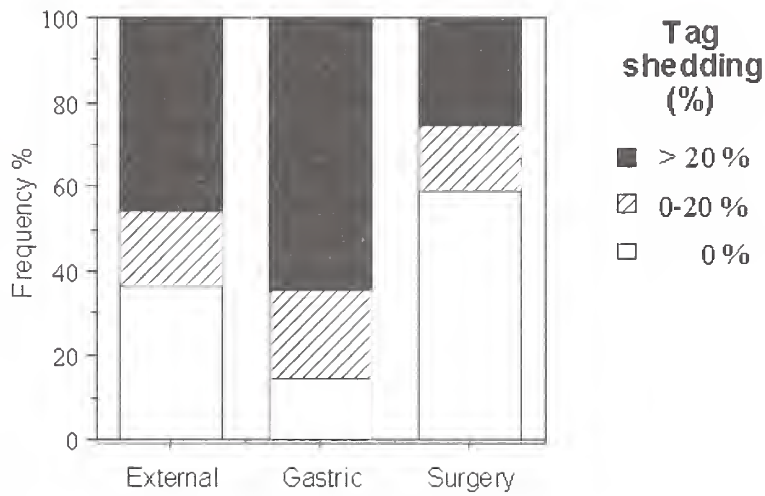

Figure 7.3. Proportion of telemetry studies reporting variable rates (0 %, < 20 %, 20 %) of tag shedding depending on attachment procedure.

a) Shedding of externally-attached tags

Externally-attached transmitters can be programmed to be shed by fish on purpose, by using absorbable attachment threads such as catgut, or by use of pop-up technology (Block et al 1998; Lutcavage et al. 1999). Tags fixed by non-absorbable threads are supposed to remain attached to the body of the fish, but shedding has been frequently reported (Figure 7.3), as exemplified by tags attached at the base of the anal fin of yellowtail, *Seriola quinqueriadata*, that were shed on average 8 days after tagging (Ichihara *et al.*, 1972), or by tags attached dorso-laterally to lake whitefish, *Coregonus clupeaformis* (Bégout *et al.*, 1998). The main causes invoked were untied knots (e.g. barbel, *Barbus barbus*, Baras, 1992; dace, *Leuciscus leuciscus*, Beaumont *et al.*, 1996) or deep cuts

in the dorsal musculature caused by attachment wires (e.g. lake whitefish, Bégout *et al.*, 1998) as a result of drag. The use of cyanoacrylate adhesive at the time of tagging can secure knots. Attachment plates frequently used in side-saddle harnesses reduce the extent of cuts and subsequent shedding rates (e.g. < 5 % after three months in yellow perch, *Perca flavescens* and < 5 % after 37 d in black bass, *Micropterus salmoides*; Ross & McCormick, 1981; 0 % after 45 days in white perch, *Morone americana* and rainbow trout, *Oncorhynchus mykiss*, Mellas & Haynes, 1985). However, harnesses may cause erosion of scales and muscles in the long run and eventually promote microbial infection and death of tagged fish. Similarly, more secure knots may untie later, and possibly at different times, and thus cause the fish to drag the tag at the extremity of the attachment wire (Beaumont *et al.*, 1996). This almost certainly modifies fish behaviour. Feasibility studies with externally-attached transmitters have rarely lasted more than 90 days, and it is thus uncertain whether tags may be retained for long periods, especially for side-saddle harnesses, which may strongly interfere with growth, and cause deep cuts to the fish musculature.

(b) Regurgitation and egestion of gastrically-inserted tags

Transmitters in bait that are voluntarily ingested by fish have never been reported to damage the digestive tract of the fish, whereas damage to the oesophagus was observed when transmitters were inserted with a plunger (McCleave & Horrall, 1970; Solomon & Storeton-West, 1983). Stomach-inserted or ingested transmitters may be lost through regurgitation (vomiting) or egestion (defecation). Regurgitation rates and delays between ingestion (or insertion) and regurgitation vary greatly, depending on the fish species and the relative size of the tag (Moser *et al.*, 1990; Nielsen, 1992). Regurgitation rates generally increase as relative tag size increases (Nielsen, 1992). Small tags, in contrast, may be lost through egestion (Mortensen, 1990; Baras, 1992). Some species are known to regurgitate transmitters more frequently than others (Table 7.1). Recently, Marmulla & Ingendahl (1996) suggested that the mode of capture influenced the propensity of salmonids to regurgitate tags: sea trout captured with electric fishing in rivers regurgitated sooner and more frequently than those captured by netting.

Table 7.1. Fish species with high and low potential for retaining gastrically-inserted transmitters. (after Nielsen, 1992; adapted from Stasko & Pincock, 1977, and others).

Regurgitation unlikely	Regurgitation likely
Alosa sapidissima;(American shad)	*Catostomus commersonni*;(white sucker)
Anguilla rostrata;(American eel)	*Esox lucius*;(Northern pike)
Ictalurus nebulosus;(brown bullhead)	*Gadus morhua*;(Atlantic cod)
Morone chrysops;(white bass)	*Katsuwonus pelamis*;(skipjack tuna)
Morone saxatilis;(striped bass)	*Oncorhynchus kisutch*;(coho salmon)
Oncorhynchus gorbuscha;(pink salmon)	*Oncorhynchus mykiss*;(rainbow trout)
Oncorhynchus keta;(chum salmon)	*Perca flavescens*;(yellow perch)
Oncorhynchus nerka;(sockeye salmon)	*Salmo salar*;(Atlantic salmon)
Oncorhynchus tshawytscha;(Chinook salmon)	*Salmo trutta*;(brown trout)
Salvelinus namaycush;(lake trout)	*Stizostedion canadense*;(sauger)
Thunnus thynuus;(bluefin tuna)	

c) Expulsion of surgically-implanted transmitters

By contrast with terrestrial vertebrates, fish maintain near-neutral buoyancy. They have not developed their abdominal region to cope with gravity effects like these induced by negatively-buoyant transmitters or tags, and this may account for the relatively frequent expulsion of implants by fish. Early implant exit may take place through the incision before healing is completed and is generally a consequence of loose suturing. Implants may be expelled later, either through the incision, through an intact part of the body wall, or through the intestine (channel catfish, *Ictalurus punctatus*, Summerfelt & Mosier, 1984; rainbow trout, *Onchorhynchus mykiss*, Chisholm & Hubert 1985; Lucas, 1989; Atlantic salmon smolts, *Salmo salar*, Moore *et al.*, 1990; vundu catfish, *Heterobranchus longifilis*, Baras & Westerloppe, in press). All three modes of exit share a common mechanism, which consists of the encapsulation of the implanted tag by proliferating granulation tissue consisting of collagen and myofibroblasts (Marty & Summerfelt, 1986, 1990). The contraction of myofibroblasts adds to the gravity pressure exerted by the transmitter over the fish tissue, and forces the implant through the route of least resistance. During the transintestinal expulsion process, the implant capsule adheres to at least two points of the intestinal peritoneum, as well as to the parietal peritoneum. The resulting rigidity interferes with the movements of the intestine during digestion and causes the dislocation of the muscular layer of the pyloric intestine, allowing the implant to pass into the lumen of the intestine and thence to be transported by reflex peristalsis to the anus.

Encapsulation is a classical body reaction and has been observed with all coatings assayed to date, and this suggests that the expulsion process is not specific to coating (Baras *et al.*, in press). Further, no anal or body wall exit was observed in some species like blue tilapias (*Oreochromis aurens*) which encapsulated implants almost systematically (Thoreau & Baras, 1997). It is worth emphasising that not all fish species encapsulate tags, and the propensity for expulsion is thus species-dependent, especially for transintestinal expulsion, which seems specific to siluriform species (channel catfish, *Ictalurus punctatus,* Marty & Summerfelt, 1986; vundu catfish, *Heterobranchus longifilis*, Baras & Westerloppe, in press). Factors that promote the expulsion of implanted tags include the position of the tag and tag:fish size ratios. Positioning the implant far from the incision, either through a plunger or using a shielded needle technique, minimises the risk of pressure over this weakened tissues and promotes long term retention of the implant (Ross & Kleiner, 1982; Baras & Westerloppe, in press). Incidence of rejection of transmitters through the body wall, or incision site, seems to increase with transmitter size (channel catfish, *Ictalurus punctatus,* Summerfelt & Mosier, 1984; Chisholm & Hubert, 1985; Marty & Summerfelt, 1986; rainbow trout, *Onchorhynchus mykiss*, Chisholm & Hubert 1985). Large transmitters are, however, less likely to enter the intestine and be expelled by peristalsis (Lucas, 1989; Baras & Westerloppe, in press). Bleeding during surgery favours the formation of clots and adhesions (Rosin, 1985) which are involved in the encapsulation and expulsion processes. Similarly, factors that promote the invasion of the body cavity by microbial organisms, such as external whip antennas of radio transmitters, or permanent suture materials, also increase the risk of expulsion (Baras *et al.*, in press). In this respect, braided suture filaments were recently shown to cause more frequent transintestinal expulsion in vundu catfish, *Heterobranchus unifilis,* than monofilaments (Baras & Westerloppe, in press), essentially because the former provide a larger surface for the settlement of micro-organisms than the latter. Prophylaxis and use of antibiotics may thus be extremely advantageous to minimise or prevent tag expulsion.

Although transmitter loss is undesirable scientifically, it should be noted that transmitter expulsion does not necessarily lead to subsequent mortality or morbidity (channel catfish, *Ictalurus punctatus,*

Marty & Summerfelt, 1986; rainbow trout, *Onchorhynchus mykiss*, Lucas, 1989; Atlantic salmon smolts, *Salmo salar*, Moore *et al.,* 1990; vundu catfish, *Heterobranchus longifilis*, Baras & Westerloppe, in press).

7.4.4. Infections and wounds

Fish with externally-attached and surgically-implanted transmitters may have infections and wounds at the attachment points and the incision (e. g. yellow perch, *Perca flavescens*, Ross & McCormick, 1981; white perch, *Morone americana*, Mellas & Haynes, 1985; barbel, *Barbus barbus*, Baras, 1992; bluegill, *Lepomis macrochirus*, Knights & Lasee 1996; European eel, *Anguilla anguilla*, Baras & Jeandrain, 1998). In freshwater, especially at higher temperatures, fungus infection may be a problem, especially for salmonids (rainbow trout, *Onchorhynchus mykiss,* Lucas 1989; Kaseloo *et al.,* 1992; Chinook salmon, *Onchorhynchus tshawytscha*, Adams *et al.,* 1998), but these infections are not specific to external wounds, since they were also observed in salmonids with gastrically-inserted transmitters, possibly as a consequence of handling (Solomon & Storeton-West, 1983). Infections are enhanced by the presence of permanent transcutaneous bodies (Roberts *et al.*, 1973) such as the threads of externally-attached transmitters, permanent suture material or externally trailing antennas of radio tags. Similar problems are also encountered frequently for gastrically-inserted transmitters with trailing antennas that cause abrasion of the mouth corner (e.g. Chinook salmon, *Onchorhynchus tshawytscha*, Martinelli *et al.,* 1998). Threads of external transmitters or heavy tags, as well as suture materials, can also cause deep cuts into the muscles and skin (yellowtail *Seriola quinqueradiata* Ichihara *et al.,* 1972; barbel, *Barbus barbus*, Baras, 1992; lake whitefish, *Coregonus clupeaformis*, Bégout *et al.,* 1998). These cuts promote further infection of the fish by microbial organisms (bluegill, *Lepomis macrochiris*, Knights & Lasee, 1996), or cause the tissue to become necrotic and prevent normal healing (rainbow trout, *Onchorhynchus mykiss,* Kaseloo *et al.,* 1992; bluegill, *Lepomis macrochiris,* Knights & Lasee, 1996; European eel, *Anguilla anguilla*, Baras & Jeandrain, 1998). Fast flowing environments, which increase the drag of externally-attached tags, can cause abrasion of the skin beneath the tag, or the foam pad on the side of the fish. These abrasions can eventually cause microbial invasion (white sucker, *Catostomus commersoni*, Lonsdale & Baxter, 1968; yellow perch, *Perca flavescens*, Ross & McCormick, 1981; hybrid bass Yeager, 1982; barbel, *Barbus barbus*, Baras, 1992; Atlantic cod, *Gadus morhua*, Thorsteinsson, 1995; sea bass, *Dicentrarchus labrax*, Claireaux & Lefrançois, 1998). The severity of wounds is often worse in cryptic or highly structured environments, in which externally-attached tags can become entangled in surrounding vegetation, or torn by rocky substrata (yellow perch, *Perca flavescens*, Ross & McCormick, 1981; Atlantic salmon, *Salmo salar* smolts, Nettles & Gloss, 1987; Chinook salmon, *Oncorhynchus tshawytscha*, Adams *et al.*, 1998).

Internally positioned transmitters can cause wounds too, either during inserting, or later, as a result of movements of the tag inside the fish. Plungers used to insert intragastric tags may damage the stomach or the oesophagus (cutthroat trout, *Oncorhynchus clarki*, McCleave & Horrall, 1970; sea trout, *Salmo trutta*, Solomon & Storeton-West, 1983). Scalpels may puncture viscera or ovaries, especially when making incisions laterally to the midventral line (grass carp, *Ctenopharyngodon idella*, Schramm & Black, 1984; Baras *et al.,* in press). Surgically-implanted transmitters may move inside the body cavity and cause various types of damage such as alterations to gonads (Chamberlain, 1979), internal haemorrhages (rock bass, *Ambloplites rupestris*, Bidgood, 1980; carp, *Cyprinus carpio*, Otis & Weber, 1982; Mortensen, 1990), bruised livers or erosion of the rectum (grass carp, *Ctenopharyngodon idella*, Schramm & Black, 1984), necrosis of the pelvic girdle (bluegill, *Lepomis macrochirus*, Prince & Maughan, 1978) or rupture of the body wall or intestine prior to expulsion (channel catfish, *Ictalurus punctatus*, Marty & Summerfelt, 1986; rainbow trout,

Oncorhynchus mykiss, Lucas, 1989; vundu catfish, *Heterobranchus longifilis*, Baras & Westerloppe, in press). Attempts have been made to suture implanted transmitters to the body wall in order to prevent movement inside the body cavity and consequent damage to viscera. However, these attempts have produced highly variable results depending on species. Petersen & Andersen (1985) succeeded while tagging Atlantic cod, *Gadus morhua*, whereas transmitters sutured to the body wall of channel catfish *Ictalurus punctatus* were almost systematically expelled (Marty & Summerfelt, 1986).

Most damage can be prevented, or alleviated, by tailoring the attachment procedure to the species of interest and prevailing environmental conditions. Adjustments include tag size, shape, length and coating, tag positioning, attachment threads (external tags), incision site and closing material (intraperitoneal tags), and use of appropriate prophylactic measures (see Summerfelt & Smith, 1990; Baras *et al.*, in press).

7.4.5. Effects on growth and feeding

Depressed growth rate, or weight loss of fish has been observed frequently after tagging, but with variable extent and duration, depending on fish species, life stage and attachment procedure. Growth is an integrating variable of fish physiology and behaviour, and impaired growth may thus be the consequence of habitat change, depressed mobility or competitive ability, difficulties in recovering buoyancy, change of social status, increased energy expenditures or reduced appetite.

The degree of stomach fullness is a well-known factor that regulates the appetite of fishes. Feeding can be terminated by a full stomach (Toates, 1981; Jobling, 1994) and gastrically-inserted tags may induce similar reactions. The problem does not arise with adult salmonids and other species that do not feed during spawning migrations. Tags affect food intake in proportion to the tag:fish weight ratio, although it seems likely that this effect is governed by the relative volumes of tag and stomach. Moser *et al.* (1990) observed that tag ratios less than 4.5 % did not affect feeding and growth of juvenile coho salmon, *Oncorhynchus kisutch* whereas higher ratios (4.5-14.5 %) reduced the feeding rate. Similarly, Armstrong and Rawlings (1993) reported that Atlantic salmon (*Salmo salar*) parr did not feed after the insertion of transmitters into their stomachs. Adams *et al.* (1998) and Martinelli *et al.* (1998) observed that gastrically-inserted transmitters averaging 4 and 6% of the body weight of juvenile Chinook salmon, *Oncorhynchus tshawytscha,* impaired their growth over longer periods than tags inserted into the peritoneum. However, not all species seem to be affected in the same way, since the food intake of Atlantic cod, *Gadus morhua,* was not modified after gastric-insertion of transmitters (Reference needed). Whether abrasion of the corner of the mouth, which is frequently observed in fish tagged with transmitters involving the external antenna trailing from the mouth (e.g. Chinook salmon, *Oncorhynchus tshawytscha*, Martinelli *et al*., 1998), affects the feeding rate or growth of the fish, is uncertain.

No long term effects on feeding and growth have been found in studies with surgically-implanted transmitters in muskellunge (*Esox masquinongy*; Crossman, 1977), channel catfish (*Ictalurus punctatus*; Summerfelt & Mosier, 1984), Colorado squawfish (*Ptychocheilus lucius*; Tyus, 1988), razorback sucker (*Xyrauchen texanus*; Tyus, 1988), juvenile Atlantic salmon (Moore *et al.,* 1990) and rainbow trout (Lucas, 1989, Martin *et al.,* 1995). However, studies where growth was investigated at shorter time intervals provided evidence that the growth of surgically-tagged barbel (*Barbus barbus*; Baras, 1992), vundu catfish (*Heterobranchus longifilis*, Baras & Westerloppe, in press) or blue tilapia (*Oreochromis aureus*; Thoreau & Baras, 1997) was impaired over the first few

post-tagging days, but was then compensated for by higher than normal growth rates. Growth rate returned to normal again when the surgical incisions had healed. Factors invoked included partly excessive tag ratios that restricted access to food resources, or feeding subordinated to untagged individuals that appeared dominant at feeding time (bluegill *Lepomis macrochirus*, Knights & Lasee 1996). During the transintestinal expulsion process in catfishes, tags may also cause a transient blockage of food, of which the duration is uncertain, but is apparently long enough to depress the growth of the fish.

The effects of external tags on feeding and growth rate have also been investigated, but essentially during short or mid-term feasibility studies. No effects were found in yellow perch (*Perca flavescens*; Ross & McCormick, 1981), dace (*Leuciscus leuciscus*; Beaumont *et al.,* 1996) or lake whitefish (*Coregonus clupeaformis*; Bégout-Anras *et al.*, 1998), whereas externally-tagged largemouth bass (*Micropterus salmoides*) showed lower predation rates on minnows (Ross & McCormick, 1981), and barbel (*Barbus barbus*) carrying external dummy tags lost weight over several weeks after tagging (Baras, 1992). Similarly, the feeding rates and growth in parr of Atlantic salmon (*Salmo salar*) was affected by external tagging, and growth impairment was proportional to the tag ratio (Greenstreet & Morgan, 1989). In contrast to intraperitoneally-implanted transmitters, the effects of external tags on growth and feeding may be progressive and increase in the long run, essentially because of permanent wounds, and generally deeper cuts to the musculature as time goes by. Side-saddle harnesses are also deemed to interfere mechanically with the growth of the fish but no study has evaluated this problem over long periods.

7.4.6. Effects of tags on behaviour

The effects of tags and tagging procedure on fish behaviour or physiology have been relatively poorly documented, essentially because these aspects have rarely been investigated during feasibility studies (see Figure 7.1). Reasons for this include the difficulty of measuring physiological variables accurately in live fish without causing additional interference, and the discrepancy between experimental environments used in feasibility studies and wild environments. Furthermore, changes in behaviour can be more discrete and last for shorter periods of time, and thus be far less obvious to detect than mortality, tag shedding or reduced growth.

(a) Buoyancy and posture

With few exceptions (e.g. tunas or catfishes), teleost fish maintain reduced body density by adjusting the volume of their swim bladder. Many fish with swim bladders are negatively buoyant over much of the water column, only approaching neutral buoyancy at the top of their vertical range (Blaxter & Tytler, 1978; Harden Jones & Scholes, 1985; Arnold & Greer Walker, 1992). The swim bladder is said to have a volume of about 5 % of fish volume in marine fishes, and about 7 % in freshwater fishes, though these are theoretical values and real data are much more variable. More importantly, the swim bladder has an adjustment capacity of about 25 % (Alexander, 1966; Bone & Marshall 1982). This adjustment capacity permits the fish to cope with increased mass, such as that caused by negatively-buoyant eggs or tags. Physostomatous fish such as salmonids or anguillids possess a connection between the swim bladder and the gut, and can refill their swim bladder by swallowing air. The connection is absent in the vast majority of teleosts (physoclistous fish), in which gas exchange takes place via the *rete mirabile* (Bone & Marshall, 1982).

This anatomical difference implies that physostomes can regain near-neutral buoyancy more rapidly than physoclists after attachment of a negatively-buoyant transmitter or DST, provided they can access the surface (e.g. Atlantic salmon, *Salmo salar*, Fried *et al.*, 1976). Physoclistous percids remain on the bottom until sufficient gas is secreted, whereas cichlids or centrarchids like the bluegill (*Lepomis macrochirus*) increase their fin beat frequency to create the upward force necessary to reach shallow depths where they can achieve neutral buoyancy (Gallepp & Magnuson, 1972). Similarly, blue tilapia *Oreochromis aureus* take about 72 hours to compensate for the negative buoyancy and slight postural disequilibrium caused by implantation of a transmitter which adds 0.9 % to their body mass (Thoreau & Baras, 1997). Swimming compensation may also take place in physostomatous fish denied access to the surface (Fried *et al.*, 1976), and in negatively-buoyant fish like scombrids or thunnids, which swim continuously to avoid sinking and for which adding weight implies faster swimming.

Tagging thus imposes temporary or permanent constraints on fish bioenergetics, of which the energetic cost has rarely been quantified, but is presumably directly proportional to the tag:fish weight ratio. This accounts partly for the observation that most fish carrying tags representing more than 1.75-2.00 % of their body weight in water show deviant behaviour subsequent to tagging, whereas minimal or zero effects are observed for lower ratios (e.g. McCleave & Stred, 1975; Greenstreet & Morgan, 1989; Moser *et al.*, 1990; Kaseloo *et al.*, 1992; Voegeli *et al.*, 1998). More adverse effects of capture and release procedures can theoretically take place when fish are captured in deep water and transported to the surface for tagging, as this rapid change of depth can damage the swim bladder (see Chapter 5).

(b) Swimming performance and energetic expense

As mentioned earlier, negative buoyancy induced by tagging may cause the fish to increase its fin beat frequency to compensate for added mass, regardless of the attachment procedure. However, additional specific adverse effects may originate from the procedure itself. Externally-attached tags are usually positioned further from the centre of gravity of the fish than internally positioned tags. Because of this they are more prone to cause permanent or temporary postural disequilibrium and irregular swimming (e.g. Atlantic salmon *Salmo salar*, Thorpe, 1981; largemouth bass *Micropterus salmoides*, Mellas & Haynes, 1985; dace *Leuciscus leuciscus*, Beaumont *et al.*, 1996). Drag resistance of externally-attached tags varies depending on transmitter bulk and shape.

Swimming performance may be affected by the presence of a transmitter, which is especially important to consider when dealing with migratory species, such as salmonids, and active pelagic species, such as scombroids. Drag resistance of externally-attached transmitters is the most obvious cause of reduced swimming capacity, but large internal transmitters may inhibit swimming movements, reducing available power. Other effects of transmitters that reduce the health of the fish and/or increase the energy demand, will also combine to affect swimming performance.

Externally-tagged rainbow trout have been shown to exhibit lower exhaustion times than other tagged groups or control fish (Mellas & Haynes 1985). In another study of rainbow trout, two types of externally-attached transmitters raised both tail beat frequency (TBF) and opercular beat rate (OBR), but a transmitter consisting of two packages mounted symmetrically on either side of the body affected TBF and OBR least (Lewis & Muntz 1984). In a study of Atlantic salmon smolts, critical swimming speeds were lower in fish with external transmitters (McCleave & Stred 1975). Drag measurements of external transmitters in a flume indicated that the extra power output required for tagged plaice (*Pleuronectes platessa*) and cod (*Gadus morhua*) to maintain the same steady

speed as untagged fish was between 3 and 5 %, which in this study was considered negligible (Arnold & Holford 1978). In a field study of adult chinook salmon (*Oncorhynchus tshawytscha*), upstream migration in a river was successful in externally-tagged fish, which migrated at the same speed as control fish. In contrast, most of the fish with surgically-implanted transmitters were not able to pass a dam, and eventually migrated downstream (Gray & Haynes 1979). No effects of the transmitters on swimming performance were detected in swimming tests of juvenile Atlantic salmon with surgically-implanted transmitters, white perch (*Morone americana*) with surgically-implanted, externally-attached and gastrically-inserted transmitters, and rainbow trout (*Oncorhynchus mykiss*) with surgically-implanted and stomach-inserted transmitters (Mellas & Haynes 1985, Moore *et al.*, 1990).

Studies dealing with swimming performance of tagged fish demonstrate that the effects vary considerably. Swimming performance seems least affected when transmitter size and volume are as small as possible in proportion to fish size (e. g. McCleave & Stred 1975).

(c) Effect on social behaviour and interactions between species.

The effect of tagging or tag presence on predation risk has rarely been investigated in feasibility or field studies. Because of the difficulty in recovering neutral buoyancy, or because of reduced swimming capacities, fish tagged with electronic tags may be more vulnerable to predation than untagged fish (Jolley & Irby, 1979; Ross & McCormick, 1981; Eiler, 1990). External tags may also make tagged fish more easily detected by predators, and it is thus recommended that external transmitters are camouflaged to reduce their visibility (Ross & McCormick, 1981). Similarly, handling or tagging procedures may affect the social status of the fish. Surgically-tagged Guadeloupe bass (*Micropterus treculi*) showed less social tendencies than untagged fish (Manns & Whiteside, 1979; Manns, 1981), and externally-tagged yellowtail (*Seriola quinqueradiata*) showed depressed social behaviour over the first hour after tagging (Ichihara *et al.*, 1972). In other circumstances, tagging did not modify shoaling or schooling (e.g. Baras, 1997). With respect to species exhibiting territorial behaviour or social hierarchy, occasional changes of social status were observed in rainbow trout (*Oncorhynchus mykiss*) carrying tags in their stomach (Mellas & Haynes, 1985), whereas surgery was not enough to cause reversal of a well established hierarchy, either in brown trout (*Salmo trutta*; Baras *et al.*, in prep.) or rainbow trout (*Oncorhynchus mykiss*, Swanberg & Geist, 1997). However, similar status changes were also seen in fish that had only been handled, suggesting that this adverse effect did not originate from tagging, but from the capture and handling procedure (Baras *et al.*, in prep.). It is strongly suggested that such adverse effects on fish behaviour must also be considered when tagging fish with conventional tags or PIT tags.

Considering the various adverse effects of tagging and their dynamics, the risk of predation or change of social status is highest during the post-tagging hours or days for all attachment procedures, then vanishes when wounds have healed. Exceptions to this rule of thumb are mainly concerned with external transmitters, for which adverse effects can cumulate over time. This applies particularly to spawning behaviour, and it is generally recommended that fish are not tagged during the reproductive period (Winter, 1996). Fish are deemed to be more delicate at this time (Økland *et al.*, 1996) and there is a higher risk of damaging the enlarged gonads of females when implanting tags in he body cavity (Bidgood, 1980; Schramm & Black, 1984). However, adverse effects of tagging mature fish are not systematically observed and some species spawn successfully less than one week after abdominal surgery and transmitter implantation (Baras, 1995). Similarly, most studies where the gonadal development of fish with surgically- implanted tags has been evaluated show little or no difference from controls (Moore *et al.,* 1990, 1994; Martin *et al.,* 1995; see parallel with PIT tags in

Baras *et al.,* in press). There may even be advantages in tagging mature individuals of species like the vundu catfish, *Heterobranchus longifilis*, in which enlarged gonads may prevent transintestinal expulsion of tags(Baras & Westerloppe, in press).

(d) Mobility and habitat selection

There are a few studies of the effects of tags on mobility and habitat selection in artificial rivers (e.g. brown trout, *Salmo trutta*, Baras *et al.*, in prep.), or culture tanks (e.g. blue tilapia, *Oreochromis aureus*, Thoreau & Baras, 1997). Most tag-induced biases have, however, been reported from field studies. Irregular swimming, erratic movements and apparent disruption of surface avoidance behaviour have been reported in several species (Guadeloupe bass, *Micropterus treculi*, Manns & Whiteside, 1979; largemouth bass, *Micropterus salmoides*, Mesing & Wicker, 1986). Hypoactivity of newly tagged fish is most frequent (e.g. rainbow trout, *Oncorhynchus mykiss*, Zimmermann, 1980; blue tilapia, *Oreochromis aureus*, Thoreau & Baras, 1997), as well as increased downstream movements of upstream migrants (Chinook salmon, *Oncorhynchus tshawytscha*, Haynes & Gray, 1979). However, post-release hyperactivity has been observed too (Atlantic cod, *Gadus morhua*, Hawkins *et al.*, 1974; Lake whitefish, *Coregonus clupeaformis*, Bégout-Anras *et al.*, 1998). Further, both hypo- and hyperactivity have been observed in the same species (Thoreau & Baras, 1997), and this makes it difficult to determine whether these were just normal changes in the activity level of the fish, or actual perturbations resulting from the tagging procedure. Similarly, both upstream and downstream movements were observed in sick brown trout, *Salmo trutta,* that died eventually, and long downstream movements were observed in healthy individuals (M. Ovidio, unpublished data).

This variability considerably limits the relevance of behavioural criteria, essentially because the behaviour of the fish prior to tagging is generally unknown. Hence it is suggested (Lagardère *et al.*, 1996; Baras *et al.*, in press) that these criteria would be best used within a framework of individual modes, for an *a posteriori* determination of when the fish stopped behaving normally.

(e) Additional perturbations of behaviour

The use of electronic tags in fisheries is deemed to minimise the subsequent stress of recapture that is frequently encountered in conventional tagging studies. However, radio or acoustic telemetry frequently implies that the fish is tracked from the banks of a river, or from a tracking boat in lakes or at sea, and this may cause temporary perturbations of fish behaviour. Vibrations on river banks during tracking can cause fish to move away from the noise source, or to dive in deeper water (Baras, unpublished). Similar behaviour was reported for European eels (*Anguilla anguilla*); these do not change swimming direction, but dive to greater depth when a boat approaches within 10 m, then regain their original depth after the boat has passed (Westerberg, 1983). Boat engines are extremely noisy and can be detected at distances of hundreds of meters by several fish species, including Atlantic cod *Gadus morhua* (Stasko & Buerkle, 1975). Whether all fish change their mobility pattern at the approach of a boat is uncertain. Stasko & Pincock (1977) stated that pink salmon (*Oncorhynchus gorbuscha*), Chinook salmon (*O. tshawytscha*), American eel (*Anguilla rostrata*), white bass (*Morone chrysops*) and largemouth bass (*Micropterus salmoides*) were apparently not affected, while reactions had been reported frequently in dusky shark (*Carcharhinus obscurus*), white marlin (*Tetrapturus albidus*) and in some cases in sockeye salmon (*O. nerka*) and Atlantic salmon (*Salmo salar*). Avoidance reactions of marine fish to research vessels and fishing gear are discussed in some detail in Miston (1995)

7.4.7. Effects of tags on physiology

Although the physiology of newly tagged fish has rarely been investigated, one aspect of this problem has already been addressed indirectly in section 7.3.3.f., which deals with the physiological changes (i.e. increased gas exchange or increased rates of fin movement) that may be needed to compensate for the added mass of the tag.

Surgically-tagged fish with open incisions may experience difficulty in maintaining their osmotic balance, and their physiology may thus be affected for a variable period, whose length will depend on the capacity of the fish to repair tissue. This period is likely to last at least until the incision is filled with connective tissue (2 days to several weeks, depending on species, age and temperature; see Anderson & Roberts, 1975; Baras *et al.*, in press). It should be complete once the epidermis has been reconstituted over the incision area. However, these aspects have never been investigated in detail, and it is also uncertain whether quicker ways to close the incision, such as use of cyanoacrylate adhesives, minimise the problem (Nemetz & MacMillan, 1988; Petering & Johnson, 1991; Baras & Jeandrain, 1998). Similarly, the effects of chronic lesions caused by the threads of external tags on osmotic balance are unknown.

There is little doubt that infections, haemorrhages or damage to organs due to erosion by the tag, or the tag expulsion mechanism, affect fish physiology too, but the extent of these perturbations has rarely been measured during tagging feasibility studies. Martinelli *et al.* (1998) provided evidence for reduced levels of plasma proteins in newly tagged Chinook salmon (*Oncorhynchus tshawytscha)* that lasted for at least 5 days in surgically-tagged fish, and at least 21 days in fish carrying transmitters in their stomachs. These changes were deemed to reflect reduced food intake. Claireaux and Lefrançois (1998) measured metabolic rates of externally-tagged Atlantic cod (*Gadus morhua)* and sea bass (*Dicentrarchus labrax)* and found that these were substantially higher than in untagged fish, although they estimated that the impact of tag carrying was low with respect to the metabolic capacities of these two species.

7.4.8. Effects of PIT tags

Because of their small size (11 x 2.2 mm in diameter, 70 mg in the air and 40 mg in water), there is a low probability that PIT tags cause a major interference with fish life processes (Nielsen, 1992), and this is indeed the case in husbandry management programmes where the technique is used (Jenkins & Smith, 1990; Poncin *et al.*, 1990). Short term effects of PIT tagging have been noticed while tagging broodstock, but these are mainly a result of capture and handling (Baras & Westerloppe, in press).

However, precisely because of their small size, PIT tags can be applied to small juvenile fish (Prentice *et al.,* 1990; Peterson *et al.,* 1994; Ombredanne *et al.,* 1998), which may thus be confronted with problems similar to those encountered in telemetry studies with adult fish, where transmitters are implanted into the body cavity. These include difficulties in buoyancy compensation, reduced access to food and slower growth over the first post-tagging days when using tag ratios above 3 % in the air (Nile tilapia, *Oreochromis niloticus*; Baras *et al.*, in press; perch, *Perca fluviatilis*; Baras *et al.,* submitted). Similar but less severe effects were noticed in fish with lower tag ratios (Baras *et al., op cit.*; Baras & Westerloppe, in press). Ombredanne *et al.* (1998) also reported depressed growth of brown trout (*Salmo trutta*) parr after PIT tagging, but the extent of growth depression was comparable with that observed after adipose fin clipping alone. As for most other tags implanted

surgically, normal growth resumes when the incision has healed. Healing is usually achieved in less than 14 days (salmonids; Prentice *et al.,* 1990), and sometimes as fast as 7 days (catfishes; Baras & Westerloppe, in press), either because the incision is small compared with those used for telemetry tags, or because the fish are younger and have greater capacity for wound repair. In contrast to salmonids, the healing rate in small juvenile perch and tilapia is faster when the PIT tag is inserted manually through an incision made with a scalpel than when using conventional injectors (Baras *et al.,* in press). The latter procedure also causes much higher mortality rates than the former, and this contrasts too with young salmonids, for which injectors are usually efficient and innocuous. The relative inadequacy of injectors in tilapia or perch smaller than 10 g is due to the difficulty of controlling the penetration of the hypodermic syringe following piercing of the body wall. This is much more rigid than in salmonids, for which the injector was originally developed.

PIT tags are encapsulated in inert glass, which has few adverse effects on fish tissues, even several years after implantation. Plastic tips covering PIT tags further limit their propensity to migrate through muscular tissues, causing further damage. Probably for these reasons, the retention of PIT tags is usually extremely high (92-96 % in juvenile snapper, *Pagrus auratus*, Quartaro & Bell, 1992; 96.6 % in juvenile *Salmo trutta*, Ombredanne *et al.,* 1998; 99-100 % in Chinook salmon, *Oncorhynchus tshawytscha*, Prentice *et al.,* 1990; 100 % in largemouth bass, *Micropterus salmoides*, Harvey & Campbell, 1989). By analogy with observations in studies where sutured and non sutured incisions were evaluated (Baras *et al.,* in press; Baras *et al.*, submitted), it is likely that most tags were lost via the incision before the wound had healed. As observed for telemetry transmitters, PIT tags remained free in the body cavity of some species (Salmonids: Prentice *et al.,* 1990), whereas they frequently became encapsulated in others (Cichlids; Baras *et al.,* submitted a; Percids; Baras *et al.,* submitted b; Clariids; Baras & Westerloppe, in press). Though encapsulation was frequent in these species, no single tag expulsion was observed in juvenile tilapia or perch, at least when the incision had been closed by a single stitch. Some catfishes, however, expelled the tag through the intestine, as observed for electronic tags in adults (Baras & Westerloppe, in press).

Effects of PIT tags on physiology and behaviour have rarely been investigated. Jenkins & Smith (1990) found no adverse effect of PIT tagging on spawning in breeders of red drum (*Sciaenops ocellatus*) and striped bass (*Morone saxatilis*), and PIT tagging juvenile tilapia did not prevent their sexual maturation and breeding (Baras *et al.,* in press). Similarly, no difference was observed between the development of gonads or accumulation of abdominal lipid reserves in PIT tagged and untagged juvenile perch (Baras *et al.,* submitted b). No effect on swimming stamina or stride efficiency was found in PIT tagged juvenile Chinook salmon and rainbow trout (Prentice *et al.,* 1990), but signs of negative buoyancy were observed in juvenile perch and tilapias where tag ratios were higher than 3 %.

7.5. CONCLUSIONS

1) Tagging fish with electronic tags can generate numerous biases, the extent of which and duration of varies between species and environments. However, successes have been associated with attachment procedures tailored to the species of interest during the course of feasibility studies.

2) Scientists using electronic tags are increasingly selecting surgical techniques, mainly because adverse effects decrease over time. Surgery, however, involves longer training and more practice than is required for other attachment procedures.

3) In all tagging studies, attention should be paid to the size of the tag since excessive added weight is the most widely cited adverse bias. The tag:fish weight ratio should be kept low and drag, too, should be minimised when external tags are used. Research programmes should also be tailored to the capacities of the fish instead of imposing constraints that cannot be overcome by the fish, except after an adaptive process, whose duration exceeds that of the study.

4) Fish species have anatomical, physiological and behavioural peculiarities that make them unique, and it is thus worthwhile designing a feasibility study before implementing any field research, both for animal welfare reasons and reliability of results.

5) Increasing attention should be dedicated to lesser studied factors, such as attachment threads, closing material, tag shape and coating, pre- and post-operative care and confinement, since these may condition the actual success of tagging, and duration of post-tagging perturbation.

6) Identifying the duration of the post-operative perturbation is a sensible goal in any feasibility study, especially since electronic tags can now be programmed to transmit or collect data after delayed starts. DSTs can also be used to record post-operative effects, and thus observe directly how long the process lasts.

7.6. EFFECTS OF TAGS ON ORGANISMS OTHER THAN FISH

An exhaustive review of this topic is outside the remit of CATAG, but a few points are worth making. Tagging of marine mammals and birds (particularly seals and penguins) is common. Metal flipper tags are usually used for identification and are attached without anaesthetic. Tagging by hot-iron branding is still extensively used (e.g. on elephant seal pups; Feydak, personal communication). Though frowned upon ethically or for reasons of animal welfare, it is an extremely useful technique because the brands are readable after many years, whereas metal tags are lost. Satellite tags have been applied to both seals and penguins and are usually attached to fur or plumage by adhesives. This involves anaesthesia in seals (because they cannot be conveniently and safely immobilised in any other way). This anaesthesia may involve double administration of anaesthetics, first by darting to capture the animal concerned, secondly by administration of spinal anaesthesia during the tag attachment process. Care has to be taken to ensure that darted animals do not reach the water before capture; drowning is a significant risk.

Marine turtles have largely been tagged with flipper tags for identification. Tag loss rates are high and holes in flippers made during tagging may be susceptible to fungal infection. Satellite tags have also been attached to sea turtles. These cause minimal problems for the hard-shelled green, loggerhead, ridley and hawksbill turtles, other than increasing drag resistance (and presumably energy expenditure), but there are special problems with the large leatherback turtle *Dermochelys coriacea*. Satellite tags cannot be attached by adhesives because of the leathery, oily nature of the carapace and plastron. Early trials with towed tags, or tags attached by webbing harnesses failed with some mortalities (unacceptable in an endangered, protected species). Current satellite tagging with this species involves the fitment of plastic-protected harnesses with biodegradable portions that allow the harness to be lost after some weeks or months.

Crustaceans have been tagged at least since the 1930s. Originally, tags were attached to crabs and lobsters to establish distances of migration and metal (later plastic) tags were simply wired through holes in the shell. These holes often enlarged and showed signs of infection. The main problem for crustacean tagging is to attach a tag that remains on the animal when it molts. Wired tags had to be very carefully placed along molt lines on the carapace to achieve this. Modern tags (lobster tags, spaghetti tags, streamer tags) are generally attached to the animal by piercing muscles, often with barbed anchors, or passing tags through the abdominal musculature from one side to the other (See Chapter 4 for more detail). Access is through arthrodial membranes, not the hard shell. If this is done effectively, the tags usually survive molting. However, there are some reports of growth after molt being distorted by poorly-placed tags. No welfare problems have been reported from lobster stock enhancement programmes involving injection of coded-wire tags into the tail musculature of juvenile lobsters.

7.7. REQUIREMENTS AND RECOMMENDATIONS

All tagging procedures should aim at minimising short-term pain and stress to fish, and should avoid, as far as possible, causing long-term deterioration in health.

Planning of new tagging trials on familiar species should always involve full consideration of existing data on procedures, to ensure that mortalities, ill-health and tag losses are minimised. Laboratory feasibility studies to establish effective procedures on new species should ideally precede full field trials.

Fish tagging practitioners should all be required to undergo training Current legislation often requires experimentation license holders to undergo generalised training in the legality of various procedures and holding techniques, but surgical procedures on fish are very different from those used on terrestrial mammals.

Anaesthesia should be used to minimise pain and trauma, save in circumstances where anaesthesia itself is more detrimental to fish.

All efforts should be made to avoid chemical residues associated with the tagging process reaching the human food chain.

Discussions amongst CATAG participants suggest that low temperatures may be effective in having an anaesthetic-like effect, at least in some fish species. Where such procedures are legal, it has sometimes been found that survival of surgical procedures is better when fish are kept cold during surgery than if they are anaesthetised. It is recommended that research (including neurophysiological investigations) be carried out to evaluate whether lowered environmental temperature is a humane approach to support of tagging operations involving surgery. It is appreciated that such research would have to encompass warm-temperate fish, as well as cold-water species. In addition, the long-term consequences of cold-exposure would also require study.

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7.9. APPENDIX I.

Summary of main results from studies dealing with effects of ultrasonic and radio transmitters on fish. (http://www.hafro.is/catag/f-health&welfare/studies-res_2.htm)

7.10. APPENDIX II. Downloadable information sheets

Description of the ideal anaesthetics

(modified after Marking & Meyer, 1985, in Summerfelt & Smith, 1990)

a) Induction < 15 min, and ideally < 3 min
b) Recovery < 5 min
c) No toxicity for fish, and large tolerance margins for concentration
d) No persisting effect on fish physiology and behaviour
e) Fast excretion and/or catabolism, leaving no residues in fish tissues
f) No acclimatory or cumulative effects
g) No danger for operators
h) Easy preparation
i) Low Cost

Indicative list of the cost (1998 levels) of the main anaesthetics used in fish tagging. The cost of 1 litre of anaesthetic solution is calculated for cyprinid species at 15°C.

Compound	Presentation	Cost (ECU, VAT excl.)	Cost per litre of anaesthetic solution (ECU, VAT excl.)
Amobarbital	Powder	312 / 50 g	0.94
Benzocaine	Crystals	91 / kg	0.01
2-phenoxy-ethanol	Liquid	25 / l	0.01
Quinaldine (90 %)	Liquid	96 / l	0.03
Quinaldine sulphate	Powder	114 / 25 g	0.11
Tricaine	Crystals	180 / 100 g	0.18
Xylocaine (lidocaine)	Powder, Crystals	111 / 250 g	0.11

Tentative key for decision making when choosing between anaesthetics for fish handling and tagging

Criteria	
1.	Fish destined (C) or not destined (N) for consumption by humans
2.	Deep anaesthesia required (D) or sedation only (S, e.g. weighing)
3.	Natural environments (M), or experimental facilities, aquaculture (A)
4.	High or low volume of anaesthetic solution requested (H / L)
Anaesthetics, in decreasing order of preference	(*) = expensive, (#) = difficult to implement
CDMH:	Tricaine (stock solution)
CDML:	Tricaine (stock solution) (*), Hypothermia(#)
CDAH:	Tricaine (crystals), Hypothermia
CDAL:	Hypothermia, Tricaine (crystals) (*)
CSMH:	Tricaine (stock solution), Carbon dioxide (#), Electrical anaesthesia (DC)
CSML:	Electrical anaesthesia (DC), Tricaine (solution stock) (*), Carbon dioxide (#)
CSAH:	Tricaine (crystals), Carbon dioxide
CSAL:	Electrical anaesthesia (DC), Carbon dioxide, Tricaine (crystals) (*)
NDMH:	2-phenoxy-ethanol, Hypothermia, Tricaine (stock solution)
NDML:	2-phenoxy-ethanol, Hypothermia, Tricaine (stock solution) (*)
NDAH:	Tricaine (crystals), 2-phenoxy-ethanol, Hypothermia
NDAL:	2-phenoxy-ethanol, Hypothermia, Tricaine (crystals) (*)
NSMH:	2-phenoxy-ethanol, Quinaldine sulphate, Tricaine (stock solution), Carbon dioxide, Electrical anaesthesia (DC)
NSML:	Electrical anaesthesia (DC), 2-phenoxy-ethanol, Quinaldine sulphate, Tricaine (stock solution) (*), Carbon dioxide (#)
NSAH:	2-phenoxy-ethanol, Quinaldine sulphate, Tricaine (stock solution), Carbon dioxide, Electrical anaesthesia (DC)
NSAL:	Electrical anaesthesia (DC), Carbon dioxide, 2-phenoxy-ethanol, Quinaldine sulphate

Typical concentrations of tricaine and 2-phenoxy-ethanol recommended for deep anaesthesia

(for deep sedation about half the dose is required)

C (cold water, 5-15°C), T (temperate water, 10-25°C), W (warm water > 25°C)

Species	Family	Env.	Tricaine (mg / l)	2-phenoxy-ethanol (ml / l)
Salmo salar (Atlantic salmon)	Salmonidae	C	25	0.20-0.40
Oncorhynchus sp. (Pacific salmons)	Salmonidae	C	40-60	0.20-0.30
Gadus morhua (cod)	Gadidae	C	50	??
Thymallus thymallus (grayling)	Thymallidae	C	50-70	0.25
Oncorhynchus mykiss (rainbow trout)	Salmonidae	C	60	0.30-0.40
Salmo trutta (brown trout)	Salmonidae	C	50-75	0.20-0.30
Brycon moorei (dorada)	Characidae	W	80-100	0.40
Perca fluviatilis (Eurasian perch)	Percidae	T	90	0.40
Oreochromis niloticus (Nile tilapia)	Cichlidae	W	100	0.40
Piaractus brachypomus (colossoma)	Serrasalmidae	W	100	0.40
Prochilodus magdalenae (bocachico)	Curimatidae	W	100	0.40
Barbus barbus (barbel)	Cyprinidae	T	100	0.40
Leuciscus cephalus (chub)	Cyprinidae	T	100	0.40
Morone saxatilis (striped bass)	Percichtyidae	T	100	??
Cyprinus carpio (common carp)	Cyprinidae	T-W	100-150	0.35-0.60
Lepomis macrochirus (bluegill)	Centrarchidae	T-W	150	??
Carassius auratus (goldfish)	Cyprinidae	T-W	150-250	> 0.40
Clarias gariepinus (catfish)	Clariidae	W	120-300	0.40-0.60
Anguilla anguilla (European eel)	Anguillidae	C-T	250-500	0.80-1.00

Use of anaesthetics in fish telemetry tagging procedures

Usual name	**Tricaine**	**Exact name**	**3-amino benzoic acid ethyl ester methanesulphonate**
Synonyms:	Tricaine methanesulonate, salt of methanesulphonate, metacaine, MS-222TM, FinquelTM		
Conditioning:	- crystals highly soluble in water (1 g / 9 ml) - stock solutions short term)		
Conservation:	- Opaque bottle, stored at low temperature (crystals) - Freezing (stock solution)		
Typical concentrations:	Salmonids	25-60 mg / l	
	Cyprinids	80-150 mg / l	
	Cichlids, Characids	± 100 mg / l	
	Catfishes	100-250 mg / l	
	Eels	_ 250 mg / l	
Drawbacks:	- Affects the olfactory epithelium (channel catfish) - Acid solution, which can affect the motility of spermatozoa, and cause respiratory stress - High cost		
Toxicity	- non mutagenic - No specific toxicity at the concentrations above		
Permanence, legal aspects:	- Insignificant residues after 24 h - 21-d delay between anaesthesia and consumption (FDA)		
Suggestions	- Add sodium bicarbonate ($NaHCO_3$) before anaesthesia to buffer the anaesthetic solution (about 250 mg de $NaHCO_3$ for 100 mg of tricaine) - Do not buffer a stock solution before storage(inactivation)		

Usual name	**2-phenoxy-éthanol**	**Exact name**	**1-hydroxy-2-phenoxyetane**
Synonyms:	Ethylene glycol monophenyl ether, phenoxetol, phenoxethol, beta-hydroxyethyl phenyl ether, phenyl cellosolve		
Conditioning:	- Dense (1.1 g / l), transparent liquid, with low solubility in water (27 g / l) but high solubility in alcohol		
Conservation:	- Opaque bottle		
Typical concentrations:	Salmonids	0.2-0.4 ml / l	
	Cyprinids	0.3-0.8 ml / l	
	Cichlids, Characids	± 0.4 ml / l	
	Catfishes	0.4-0.8 ml / l	
	Eels	0.8-1.0 ml / l	
Drawbacks:	- Irritations of epithelial tissues - Little margin between induction and toxicity in salmonids		
Toxicity	- Damages the liver and kidney at subletal doses in mammals, and possibly in fish - Acute toxicity in some species		
Permanence, legal aspects:	- unknown - not approved for fish food (FDA)		
Suggestions	Prepared syringes for use in natural environments		

Usual name	**Quinaldine**	**Exact name**	**2-methylquinoline**
Synonyms:	none		
Conditioning:	- Transparent liquid, with low solubility in water but high solubility in organic solvents (alcohol, acetone)		
Conservation:	- Opaque bottle and cap (oxidation by air and light)		
Typical concentrations:	Salmonids Cyprinids Cichlids, Characids Catfishes Eels	5-12 mg / l 2,5-20 mg / l 20-40 mg / l 30-?? mg / l ?? mg / l	
Drawbacks:	- long delay between immersion and injection - fish still sensible to tactile stimuli - no action at pH < 6.0 - irritation of epithelia of operators - strong, persistent odour - strong inter individual variability of responses to anaesthesia		
Toxicity	- increases with water temperature and alkalinity - suspected as carcinogen for operators (larynx, pharynx)		
Permanence, legal aspects:	- no residue in fish muscles after 24 h - accumulation in adipose tissue - not approved for fish food (FDA)		
Suggestions	- solutions (60 % acetone, 40 % water) are highly stable, even in the long run - elimination of tactile reflexes by a preliminary injection of a relaxing compound (gallamine triethiodide, pancurorium bromide,...)		

Usual name	**Quinaldine sulphate**	**Exact name**	**Quinate**
Synonyms:	No usual synonym		
Conditioning:	- Light yellow crystalline powder, with high solubility in water		
Conservation:	- Opaque bottle and cap (oxidation by air and light)		
Typical concentrations:	Salmonids Cyprinids Cichlids, Characids Catfishes Eels	25-40 mg / l < 75 mg / l 15-60 mg / l ?? mg / l ?? mg / l	
Drawbacks:	- inconvenience typical of acid solutions (see Tricaine) - fish still sensible to tactile stimuli - irritation of epithelia of operators		
Toxicity	- increases with water temperature and alkalinity - suspected as carcinogen for operators (larynx, pharynx)		
Permanence, legal aspects:	- no residue in fish muscles after 24 h - not approved for fish food (FDA)		
Suggestions	- buffer the solution prior to use (see tricaine)		

Usual name	**Benzocaine**	**Exact name**	**Ethyl aminobenzoate**
Synonyms:	*p*-aminobenzoic acid ethyl ester, 4 aminobenzoic acid ethyl ester, ethyl-*p*-aminobenzoate		
Conditioning:	- Powder with low solubility in water but high solubility in organic solvents (acetone, alcohol)		
Conservation:	- Opaque bottle and cap (oxidation by air and light)		
Typical concentrations:	Salmonids Cyprinids Cichlids, Characids Catfishes Eels	25-50 mg / l 25-150 mg / l 25-100 mg / l ?? mg / l ?? mg / l	
Drawbacks:	- High variability of delay between immersion and induction depending on fish size and water temperature - Long recovery, especially in warm water species		
Toxicity	- increases with water temperature increase - No specific toxicity at the concentrations above		
Permanence, legal aspects:	- variability between species, accumulation in muscles - not approved for fish food (FDA)		
Suggestions	- buffer the solution prior to use (see tricaine)		

Usual name	**Carbon dioxide**	**Exact name**	**Carbon dioxide**
Synonyms:	CO_2, Carbonic acid, carbonic gas, carbonic anhydride		
Conditioning:	- non combustible gas non combustible, stored at -35°C (solid), or as sodium bicarbonate ($NaHCO_3$, powder); dissolved in water (6.75 %), with addition of sulphuric acid (3,95 %) to obtain the desired concentration in carbonic acid, at a pH in between 7 and 9		
Conservation:	- no particularity for bicarbonate - low temperature for CO_2		
Typical concentrations:	Salmonids Cyprinids Cichlids, Characids Catfishes Eels	150-650 mg / l 150-650 mg / l ?? mg / l ?? mg / l ?? mg / l	
Drawbacks:	- mainly used for sedation - risk that the operator looses conscience at _ 10 % CO_2 in the air - risk inherent to the use of sulphuric acid - risk inherent to the use of low temperature for solid CO_2 - hard to obtain deep anaesthesia, and to maintain the oxygen level		
Toxicity	- risk inherent to hypercapnia in fish, especially with respect to osmoregulation		
Permanence, legal aspects:	- No permanence - approved for fish food (FDA)		
Suggestions	mixing O_2 and CO_2 in pressurised cylinders to obtain stable concentrations		

3. REVIEW OF THE LITERATURE

3.1. GENERAL FISH WELFARE RELATED TOPICS

3.1.1. ALTERNATIVES

Altimiras J, Larsen E (2000) **Non-invasive recording of heart rate and ventilation rate in rainbow trout during rest and swimming. Fish go wireless!** *Journal of Fish Biology* 57(1):197-20
NAL Call No. QL614 J68
Resting heart rates and ventilation rates in rainbow trout *Oncorhynchus mykiss* at 15°C are 31.8 plus or minus 1.8 beat/min and 53.1 plus or minus 3.7 breaths/min, respectively. The non-invasive recording system picked up the bioelectric potentials generated by the fish in the water and was based on an array of six silver-silver chloride electrodes covered with agar-gel, which provided a better signal-to-noise ratio than in previously described systems, and allowed the determination of heart rate and ventilation rates at different swimming speeds up to 2 l s super(-1). In concert with the lower rates, the scope for changes in heart rate and ventilation rate during swimming was also considerably larger than in earlier studies (2.4- and 2.0-fold, respectively). Two main conclusions result from this work: (i) short recovery times under 48 h after anaesthesia and surgery are unlikely to provide truly resting heart rates and ventilation rates in trout at 15°C; (ii) heart rate regulation during exercise is more important than previously thought and might account for a larger proportion of the increase in cardiac output observed in swimming trout.
Descriptors: biological surveys, respiration, swimming, fish physiology, heart, *Oncorhynchus mykiss*, rainbow trout, alternative, fish
ASFA; Copyright © 2003, FAO

Dawson VK, Meinertz JR, Schmidt LJ, Gingerich WH (2003) **A simple analytical procedure to replace HPLC for monitoring treatment concentrations of chloramine-T on fish culture facilities.** *Aquaculture –Amsterdam.* 217(1-4):61-72
NAL Call No. SH1 A6
Descriptors: monitoring, model, procedure, chloramines-T, measurement, fish, alternative

Decostere A, Turnbull JF, Ducatelle R, Haesebrouck F (2000) **Development of a gill perfusion apparatus for studying the interaction of fish pathogens with gill tissue.** *ATLA, Alternatives to Laboratory Animals.* 28 (1):53-61
NAL Call No. Z7994 L3A5
The association of gill pathogens with the branchial tissue was studied using an isolated perfused gill preparation. The gill preparation consisted of an excised branchial arch from common carp (*Cyprinus carpio* L., minimum weight 300 g), perfused via the afferent branchial artery. Filtered and heparinised Cortland solution was used as the perfusion fluid and infused by means of a drip (3-litre bag). The average perfusion rate was 1.5 ml/min/arch/kg body weight. The outflowing perfusate was collected from a cannula in the

efferent branchial artery. The individual gill arch was suspended in a circular organ chamber filled with Ringer solution, which was aerated and kept at a constant temperature of 20°C. Unperfused gill arches maintained in Ringer solution at the same temperature were controls. Cortland solution proved to be a satisfactory perfusion fluid, maintaining the perfused gills in a healthy condition for at least 4 h with no, or only slight, oedema after 90 min, and slight or moderate oedema after 4 h. The unperfused gill displayed excessive necrosis and loss of architecture after 4 h. It is concluded that the Cortland perfused gill apparatus could be an alternative ex vivo model for studying early interaction of gill associated pathogens with the branchial tissue.
Descriptors: gills, disease models, necrosis, oedema, in vitro, animal welfare, fish diseases, perfusion, carp, Cyprinus, fishes

Fiksen O, MacKenzie BR (2002) **Process-based models of feeding and prey selection in larval fish.** *Marine Ecology -Progress Series.* 243:151-164
NAL Call No. QH541.5 S3M32
Descriptors: model, fish, larvae, feeding, prey selection, alternative

Fredriksson DW, Swift MR, Irish JD, Tsukrov I, Celikkol B (2003) **Fish cage and mooring system dynamics using physical and numerical models with field measurements.** *Aquacultural Engineering.* 27(2):117-146
NAL Call No. SH1A66
Descriptors: model, fish, cage, mooring, field measurement, alternative

Hernandez Molejon OG, Alvarez-Lajonchere L (2003) **Culture experiments with *Oithona oculata* Farran, 1913 (Copepoda: Cyclopoida), and its advantages as food for marine fish larvae.** *Aquaculture –Amsterdam.* 219(1-4):471-483
NAL Call No. SH1A6
Descriptors: fish, nutrition, larvae, alternative food source

Iglesias R, Parama A, Alvarez MF, Leiro J, Aja C, Sanmartin ML (2003) **In vitro growth requirements for the fish pathogen *Philasterides dicentrarchi* (Ciliophora, Scuticociliatida).** *Veterinary Parasitology.* 111(1):19-30
NAL Call No. SF810 V4
Descriptors: vitro, fish, disease, *Philasterides dicentrarchi*, parasite, growth requirements, alternative

Li L, Yakupitiyage A (2003) **A model for food nutrient dynamics of semi-intensive pond fish culture.** *Aquacultural Engineering.* 27(1):9-38
NAL Call No. SH1A66
Descriptors: model, fish, nutrition, aquaculture, nutrient dynamics, alternative

Naeve DA, Batty RS (1982) **A simple method for measuring fish larvae using silhouette photography.** *Aquaculture.* 29(1-2):165-168
NAL Call No. SH19 C53
A simple photographic technique for the rapid accurate measurement of fish larvae is described. It involves minimal handling of larvae and avoids the use of anaesthetic.
Descriptors: fish larvae, length, photography, equipment, measurement, methodology

Schmidtke LM, Carson J (2003) **Antigen recognition by rainbow trout (*Oncorhynchus mykiss*) of whole cell proteins expressed by *Lactococcus garvieae* when obtained directly from fish and under iron limited culture conditions.** *Veterinary Microbiology.* 93(1):63-71
NAL Call No. SF601 V44
Descriptors: antigen, immunologic response, *Lactococcus garvieae*, fish, iron, *Oncorhynchus mykiss*, rainbow trout

Sijm DTHM (1993) **Uptake of organic chemicals across fish gills: alternatives to the use of fish populations.** *Alternatives to Laboratory Animals.* 21 (4):453-456.
NAL Call No. Z7994.L3A5
In this study, the isolated perfused gills of rainbow trout (*Oncorhynchus mykiss*) provided a tool for fundamental research on the rate-limiting step in the uptake of organic hydrophobic chemicals. Data obtained using the isolated gills were comparable to those determined in vivo. Whereas several tens of fish need to be used to obtain statistically sound information on uptake rates in vivo, the isolated perfused gills method requires only three or four fish. A significant reduction in animal use is thus obtained.
Descriptors: rainbow trout, gills, animal welfare, animal testing alternatives, polychlorinated biphenyls, polycyclic hydrocarbons, chlorinated hydrocarbons

Suzuki K, Takagi T, Hiraishi T (2003) **Video analysis of fish schooling behavior in finite space using a mathematical model.** *Fisheries Research.* 60(1):3-10
NAL Call No. SH1F42
Descriptors: model, video, fish, schooling behavior, alternative

Zhao X, Ona E (2003) **Estimation and compensation models for the shadowing effect in dense fish aggregations.** *Ices Journal of Marine Science.* 60(1):155-163 ISSN: 1054-3139
Descriptors: model, fish, shadowing effect, alternative

Web Resources:

FADs for aquarium fish — an alternative capture method?
Lida Pet-Soede, Fini Lovita and Imam Musthofa Zainudin
http://www.spc.org.nc/coastfish/News/LRF/10/LRF10-12.htm

An Alternative Method For Sampling Small Benthic Fish In A Large Regulated River.
J.M. Howard and J.B. Layzer
http://www.benthos.org/meeting/nabs2000/nabstracts2000.cfm/id/436

Whole Effluent Toxicity (WET) Test Method Changes
Environmental Protection Agency
http://www.dnr.state.wi.us/org/water/wm/ww/biomon/Comments_on_EPA_Revisions.pdf

Sustainable Fish Farm at The Earth Centre (sustainable organic aquaculture)
http://www.fishace.demon.co.uk/index1.htm

Alternative Methods of Fish Disease Treatment
http://www.fishace.demon.co.uk/method4.htm#Alternative

3.1.2. ANESTHESIA & EUTHANASIA

Ackerman JL, Bellwood DR (2002) **Comparative efficiency of clove oil and rotenone for sampling tropical reef fish assemblages.** *Journal of Fish Biology.* 60(4):893-901
NAL Call No. QL614 J68
A quantitative examination of the reef fish assemblage at Orpheus Island, Great Barrier Reef, contrasting clove oil and rotenone, sampled 365 individuals of 47 species with clove oil v. 536 individuals of 53 species with rotenone. Number of species and individuals were not found to differ significantly between the two techniques, largely due to variation among samples. *Neopomacentrus bankieri* (Pomacentridae) and *Eviota queenslandica* (Gobiidae) were the most dominant in the samples using either technique. Although the samples appeared to be comparable, only 31 species (45 %) in eight families were common to both techniques. Fishes often recovered before collection when using clove oil and were not driven out of the reef during induction to anaesthesia. Although the samples collected with clove oil approximate the results obtained using rotenone, enclosed rotenone stations are the preferred method for providing relatively complete quantitative samples.
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Descriptors: sampling, stock assessment, fishery surveys, ichthyocides, toxicants, rotenone, marine ecosystems, coral reefs, *Neopomacentrus bankieri*, Pomacentridae, *Eviota queenslandica*, Gobiidae, Pisces, ISEW, Australia, Queensland, Great Barrier Reef, Orepheus I.
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Aguilar NG, Palcios CAM, Ross LG (1999) **Controlled anaesthesia of *Rana catesbeiana* (Shaw) and *Rana pipiens* (Schreber 1792) using xylocaine delivered by spray.** *Aquaculture Research.* 30(4):309-311
NAL Call No. SH1F8
Frog culture is an expanding industry in many parts of the world, and research on the culture of these animals has increased in recent years. This has inevitably led to a need for convenient handling and sedation, sufficient to enable various procedures to be carried out humanely. The objective of this work was to investigate the effectiveness of controlled anaesthesia using a proprietary xylocaine spray applied to the skin in two commercial species of frog, the Bullfrog, *Rana catesbeiana* Shaw, and the Leopard frog, *Rana pipiens* Schreber 1792.
Descriptors: frog culture, anaesthesia, aquaculture development, fish handling, *Rana pipiens*, *Rana catesbeiana*, Northern leopard frog, bullfrog
ASFA; Copyright © 2003, FAO

Allen JL (1988) **Residues of benzocaine in rainbow trout, largemouth bass, and fish meal.** *Progressive Fish Culturist.* 50(1):59-60
NAL Call No. 157.5 P94
Residues of the anesthetic benzocaine in muscle tissue of rainbow trout (*Salmo gairdneri*) and largemouth bass (*Micropterus salmoides*) were determined after exposure of the fish to 50 mg benzocaine/L for 15 min and withdrawal times of 0-24 h. The mean concentration of benzocaine residues in fish sampled immediately after exposure was 14.0 mu g/g in rainbow trout and 10.6 mu g/g in largemouth bass. Residues were below the control value after 8 h of

withdrawal in largemouth bass and near the control value after 4 h of withdrawal in rainbow trout. Although residues of benzocaine were high in fish immediately after exposure, the concentration declined rapidly when the fish were held in flowing fresh water. Fish meal prepared from Pacific salmon (*Oncorhynchus sp.*) that had been anesthetized with benzocaine or tricaine (MS-222) contained residues of 45.1 mu g benzocaine/g or 47.7 mu g tricaine/g.
Descriptors: anaesthetics, ethyl aminobenzoate, aquaculture, freshwater aquaculture, self purification, *Salmo gairdneri, Micropterus salmoides*, fish culture, residues, benzocaine residues
ASFA; Copyright © 2003, FAO

Allen JL, Vang G, Steege S, Xiong S (1994) **Solubility of benzocaine in freshwater.** *Progressive Fish Culturist.* 56(2):145-146
NAL Call No. 157.5 P94
Benzocaine is an effective general anesthetic for fish. Its solubility was determined in waters of various hardness and pH and over a range of temperatures (from 5 to 30°C). Variations in water hardness and pH did not appreciably affect the solubility of benzocaine, whereas increases in temperature increased solubility from 409 mg/L (at 5°C) to 1,118 mg/L (at 30°C).
Descriptors: anesthetics, freshwater fish, solubility, pH effects, temperature effects, water hardness, benzocaine
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Amend DF, Goven BA, Elliot DG (1982) **Etomidate: Effective dosages for a new fish anesthetic.** *Transactions of the American Fisheries Society.* 111(3):337-341
NAL Call No. 414.9 Am3
Etomidate (r-(+)-ethyl-1-(1-phenylethyl)-1 H-imidazole-5-carboxylate) is a potent and safe anesthetic for fish. The minimum effective dose for four species of aquarium fish zebra danio *Danio rerio*, black tetra *Gymnocorymbus ternetzi*, angelfish *Pterophyllum scalare*, southern platyfish *Xiphophorus maculatus* ranged from 2.0 to 4.0 mg/liter, and the maximum safe dose ranged from 7.0 to 20.0 mg/liter. In general, the lower the dose, the longer the time for anesthesia, but the faster the recovery time. At 4.0 mg/liter, fish typically entered anesthesia within 90 seconds and recovered within 40 minutes. Etomidate is more effective in alkaline water and higher water temperature but is not affected by total hardness. Test species varied in their ability to survive extended or repeated exposures to the drug. Etomidate has advantages over other commonly used fish anesthetics and should be evaluated further.
Descriptors: anesthetics, ornamental fish, *Danio rerio, Gymnocorymbus ternetzi, Pterophyllum scalare, Xiphophorus maculatus*, etomidate
ASFA; Copyright © 2003, FAO

Anderson WG, McKinley RS, Colavecchia M (1997) **The use of clove oil as an anesthetic for rainbow trout and its effects on swimming performance.** *North American Journal of Fisheries Management.* 17(2):301-307
NAL Call No. SH219.N66
The only anesthetic registered in North America for use in fisheries science is 3-aminobenzoic acid ethyl ester methanesulfate (tricaine or MS-222). Although MS-222 is a very effective anesthesia for several fish species, its application in the field is limited because U.S. Food and Drug Administration guidelines demand a 21-d withdrawal period after exposure to MS-222 before fish can be released and enter the food chain. As a consequence,

carbon dioxide (CO_2) has been used as a substitute anesthetic; however, induction and recovery times with CO_2 are long, and anesthesia is shallow in comparison with MS-222. We compared the efficacy of MS-222 to that of clove oil, a naturally occurring substance, for use as an anesthetic for juvenile and adult rainbow trout *Oncorhynchus mykiss*. Clove oil was as effective as MS-222 in inducing anesthesia in both age-groups. Furthermore, exposure to either clove oil or MS-222 at the concentrations tested was not detrimental to critical swimming speed of juvenile or adult rainbow trout. We propose that clove oil be considered as an alternative to MS-222 for use as a fish anesthetic.
Descriptors: swimming, anaesthetics, anesthetics, *Oncorhynchus mykiss, Eugenia aromatica*, clove oil, rainbow trout
ASFA; Copyright © 2003, FAO

Anonymous (1999) **New NZ sedative.** *Fish Farming International.* 26(3):21
NAL Call No. SH 151.F5
AQUI-S is an aquatic anaesthetic approved for use in Australia and New Zealand with a 'nil-withholding' period - meaning it is safe to use for the transportation and harvesting of food fish. Used in the handling, transportation and harvesting of farmed fish, wild fish and crustacea, its applications include grading, brood stock handling, general husbandry and rested harvesting.
Descriptors: fish handling, transportation, harvesting, aquaculture techniques, food fish, anaesthetics, Australia, New Zealand, AQUI-S, Pisces
ASFA; Copyright © 2003, FAO

Barham WT, Caiger KH, Visser JGJ (1979) **The use of benzocaine hydrochloride as fish tranquillizer and anaesthetic in saline waters.** *Journal of the Limnological Society of South Africa.* 5(2):94-96
The anaesthetic effects of various concentrations of benzocaine hydrocloride were tested on *Liza macrolepis* and *Sarotherodon mossambicus* in sea water and diluted sea water, respectively. Induction time for anaesthesia was negatively correlated with increasing anaesthetic concentrations in *L. macrolepis*. In *S. mossambicus*, however, operculum clamping appeared to be responsible for induction times increasing with increased anaesthetic concentration. The tranquillizing effects of low concentrations of benzocaine hydrochloride on *L. macrolepis* was also studied.
Descriptors: anaesthetics, saline water, Pisces, *Liza macrolepis, Sarotherodon mossambicus*, Mugilidae, Cichlidae
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Barham WT, Schoonbee HJ (1990) **A comparison of the effects of alternating current electronarcosis, rectified current electronarcosis and chemical anaesthesia on the blood physiology of the freshwater bream *Oreochromis mossambicus* (Peters). I. The effect on blood pH, pO_2, pCO_2, glucose, lactate, LDH and HBDH.** *Comparative Biochemistry and Physiology.* 96C(2):333-338
NAL Call No. QP1 C6
A comparison was made of the effects of alternating current electronarcosis, rectified current electronarcosis and the chemical anaesthesia benzocaine hydrochloride on blood physiology of the freshwater bream *Oreochromis mossambicus* over a 7 day period. A statistical evaluation of results, in particular of blood glucose and lactate levels, following narcosis, suggests that the physiological effects of alternating current electronarcosis is the least stressful of the three types of anaesthesia. It is therefore the method of choice for stress

studies as well as other studies on this fish species.
Descriptors: haematology, fish physiology, collecting devices, anaesthesia, *Oreochromis mossambicus*, comparative studies, electric currents
ASFA; Copyright © 2003, FAO

Barham WT, Schoonbee HJ (1990) **Induction behaviour of the tilapia *Oreochromis mossambicus* Peters (Pisces: Cichlidae) subjected to electronarcosis by various alternating or rectified currents.** *Water S. A.* 16(1):75-78
NAL Call No. TD319 A35W3
Mature tilapia *Oreochromis mossambicus* were subjected to electronarcosis by alternating or rectified currents at various voltages and frequencies and in water of various temperatures and conductivities and their induction behaviour observed. Responses to induction varied from no response through slight response to vigorous response. The results support earlier findings that a 60 V, 50 Hz sine wave is superior to rectified current for electronarcosis of tilapia.
Descriptors: fish culture, electric currents, electric stimuli, anaesthesia, narcosis, *Oreochromis mossambicus*
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Barham WT, Schoonbee HJ, Visser JGJ (1989) **Some observations on the narcotizing ability of electric currents on the common carp *Cyprinus carpio*.** *Onderstepoort Journal of Veterinary Research.* 56(3):215-218
NAL Call No. 41.8 On1
Some effects of alternating current electronarcosis and of rectified current electronarcosis on *Cyprinus carpio* were investigated. In all instances recovery from narcosis was accompanied by convulsive spasms. Haemorrhaging of the gills was also observed to occur. Carp do not appear to be suitable candidates for electronarcosis.
Descriptors: narcosis, fish physiology, anaesthesia, *Cyprinus carpio*, electric currents
ASFA; Copyright © 2003, FAO

Barham WT, Schoonbee HJ, Visser JGJ, Smit GL (1988) **A comparison of red-cell fragilities of electronarcotized and chemically anaesthetized freshwater bream, *Oreochromis mossambicus*.** *Comparative Biochemistry and Physiology.* 91A(2):241-244
NAL Call No. QP1 C6
Red cell fragilities were determined over a period of 7 days for a.c. electronarcotized, d.c. electronarcotized and benzocaine hydrochloride anaesthetized *Oreochromis mossambicus*. Marked differences in osmotic fragilities were apparent between the different treatments. Although a.c. electronarcosis and chemically anaesthetized fish showed only slight shifts in mean cell fragilities (MCF) with time, d.c. electronarcosis produced major shifts in this parameter after 7 days. A.c. electronarcosis and chemically anaesthesia resulted in similar fragility patterns with red cells becoming less fragile over 24 hr and subsequently returning to normal levels. The results indicate that a.c. electronarcosis compares favourably with benzocaine hydrochloride as an anaesthetic in *O. mossambicus*.
Descriptors: erythrocytes, hematology, osmosis, anesthetics, electric stimuli, *Oreochromis mossambicus*
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Bellwood DR (1981) **Cyanide..an investigation into the long term histological effects of sodium cyanide doses upon the gastro-intestinal tract of *Dascyllus trimaculatus*. Part 2.**

Freshwater and marine aquarium. 4(12):7-9,87-88
NAL Call No. SF456 F7
This part of the article investigates the distribution of cyanide throughout the fish body when cyanide is used as an anaesthetic; how and at what dose the cyanide reaches the intestine; and the influence of food in the stomach upon the movement of ingested cyanide. Cyanide induced anaesthesia is the result of severe and swift oxygen depletion in the cells, although the exact mechanism is not known.
Descriptors: digestive system, cyanides, anaesthesia, histopathology, long-term changes, *Pomacentrus violascens*
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Bernardy JA, Coleman KS, Stehly GR, Gingerich WH (1996) **Determination of benzocaine in rainbow trout plasma.** *Journal of AOAC International.* 79(3):623-627
NAL Call No. 381 As7
A liquid chromatographic method is described for analysis of benzocaine (BZ), a proposed fish anesthetic, in rainbow trout plasma. Mean recoveries of BZ from plasma samples fortified at 44-10 100 ng/mL were 96-100%. The method detection limit is 10 ng /mL, and the limit of quantitation is 37 ng/mL. Acetylation of BZ occurs in whole blood after storage at room temperature (i.e., 21°C) for 10 min. However, no acetylation of BZ was detected in plasma samples held at room temperature for 4 h. Mean method precision for plasma samples with incurred BZ residue is similar to that for fortified samples in the same concentration range (relative standard deviations of 0.9 and 1.2%, respectively).
Descriptors: haematology, anaesthetics, *Oncorhynchus mykiss*, analytical techniques
ASFA; Copyright © 2003, FAO

Bernier NJ, Randall DJ (1998) **Carbon dioxide anaesthesia in rainbow trout: effects of hypercapnic level and stress on induction and recovery from anaesthetic treatment.** *Journal of Fish Biology* 52(3):621-637
NAL Call No. QL614 J68
The physiological and anaesthetic effects of three different levels of air-saturated and buffered CO_2 anaesthesia, pCO_2 = 37, 78, or 125 mmHg, were examined in cannulated rainbow trout *Oncorhynchus mykiss*. Complete anaesthesia (no opercular movements) was not achieved by these hypercapnic levels after 20 min of CO_2 exposure. Although increasing pCO_2 reduced the induction times to the early stages of anaesthesia, it also resulted in increasing hyperventilatory, hypoxaemic, and acid-base disturbances. After a 10-min recovery period, while the respiratory acidosis component of the acid-base disturbance was corrected, there was a significant metabolic acidosis. Recovery time was longest in the high pCO_2 treatment where 33% of the fish died. Two additional groups (pCO_2 = 37 and 78 mmHg) were exposed to an acute stress prior to the anaesthetic treatment. Stress reduced the hypoventilatory effects of the low pCO_2 treatment, increased the recruitment of anaerobic metabolism, and prolonged recovery time. Although the increase in plasma catecholamines elicited by the stress was small relative to the response obtained with the anaesthetic, stress prior to CO_2 anaesthesia impaired the efficiency of the treatment. Overall, our results suggest that pCO_2 levels above 37 mmHg and/or stress prior to the anaesthesia impair the efficiency of air-saturated and buffered CO_2 anaesthesia by exacerbating the hypoxaemic effects of the hypercapnic treatment.
Descriptors: fish culture, anaesthesia, respiration, fish physiology, *Oncorhynchus mykiss*, rainbow trout
ASFA; Copyright © 2003, FAO

Billard R (1981) **Effect of Some Fish Anesthetics on Gamete Survival During Artificial Insemination of Rainbow Trout.** *Progressive Fish Culturist* 43(2):72-73
NAL Call No. 157.5 P94
Male and female fish are usually anesthetized during the process of taking spawn. They are placed in a bath with added anesthetic and removed after loss of equilibrium. This paper examines the possible toxicity of anesthetic solutions on sperm and eggs when artificial insemination is performed by the dilution technique. Of the three anesthetics (Phenoxyethanol, Quinaldine, MS-222) tested in the present experiment, only phenoxyethanol showed a toxic effect on fertilization when the concentration in the insemination diluent was higher than 0.05%. This effect was limited to the sperm; the anesthetic did not seem to affect the eggs. Some caution should therefore be exercised when this anesthetic is used to immobilize fish during spawn taking; the risk is very low because the amount of the solution contaminating the gametes is limited and unlikely to reach toxic concentrations (> 0.05%).
Descriptors: induced breeding, spermatozoa, eggs, anesthetics, toxicity, *Salmo gairdneri*

Booke HE, Hollender B, Lutterbie G (1978) **Sodium bicarbonate, an inexpensive fish anesthetic for field use.** *Progressive Fish Culturist.* 40(1):11-13
NAL Call No. 157.5 P94
Sodium bicarbonate was tested as a fish anesthetic at combinations of 142 to 642 mg/l, pH 6. 5 to 7. 5, with rainbow trout (*Salmo gairdneri*), brook trout (*Salvelinus fontinalis*), and carp (*Cyprinus carpio*). The combination of pH 6. 5 and 642 mg/l $NaHCO_3$ was the most effective treatment for causing the fish, within 5 min, to cease locomotion and slow opercular rate, but to retain reflex response to pressure on the caudal fin. It is suggested that pH-controlled carbon dioxide release from the sodium bicarbonate caused the anesthetic response.
Descriptors: fish culture, anaesthetics, sodium compounds, *Salmo gairdneri*, carbon dioxide, *Salvelinus fontinalis, Cyprinus carpio*

Bouck GR, Johnson DA (1979) **Medication inhibits tolerance to seawater in coho salmon smolts.** *Transactions of the American Fisheries Society.* 108(1):63-66
NAL Call No. 414.9 Am3
Applications of 10 therapeutic and two anesthetic agents to healthy smolts of coho salmon (*Oncorhynchus kisutch*) by conventional methods were followed by two different post-treatment circumstances. In condition 1, fish were treated and then transferred directly to 28% seawater for 10 days; in condition 2 fish were treated and held in fresh water for 4 days before their medium was gradually changed over a 4-hour period to 28% seawater. In condition 1, no mortality occurred among fish treated with 2,4-D, trichlorofon, simazine, quinaldine, or light to moderated doses of MS-222. About 10% mortality occurred among fish treated with formalin and nifurpirinol. High mortality in seawater followed treatments with copper sulphate, hyamine 1622, potassium permanganate, malachite green (one protocol), and heavy doses of MS-222. In condition 2, mortality was reduced but still high for copper sulphate and potassium permanganate, much lower for malachite green and hyamine 1622, and zero for the other agents. The results indicate that additional recovery time in fresh water is necessary between some treatments and exposure to salt water.
Descriptors: salinity tolerance, drugs, smolts, mortality causes, *Oncorhynchus kisutch*, juveniles, anaesthetics

Boulle D (1998) **The new threat looming for Indian Ocean coral reef fisheries.** *Window newsletter. Mombasa.* 9(2):1-2
ISSN: 1024-4158
Live fish capture which has been an old Chinese custom for centuries which was only practised on culture or freshwater species, involved keeping fish alive until eaten. The marine live fishery was born at the same time as the advent of cyanide fishing. This practise which is highly developed in the Western Pacific region is now making its appearance in the Indian Ocean, with operations already reported in the Maldives and Seychelles. Although cyanide fishing has two major advantages: firstly it is fast acting anaesthetic and secondly, fish exposed to cyanide quickly recover with no apparent side effects, if transferred to clean water and no residues detected in fish tissues after few days, this has lead ultimately to huge decline in coral reefs with an associated collapse of reef fishes and crustacean stocks. The logistics of transporting the fish across large distances, high demand and profit margins encourage fishing effort even after the targeted species are too rare to sustain a viable production. In an effort to safeguard the Western Indian Ocean reef fishery, managers and researchers need to be vigilant of the new developments, prepare strategies, plans and new policies that will address the problem.
Descriptors: coral reefs, reef fisheries, anaesthetics, resource conservation, fishery policy, catching methods, cyanides, stupefying methods, ISW, Indian Ocean, Southwest
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Braley H, Anderson TA. (1993) **Changes in blood metabolite concentrations in response to repeated capture, anaesthesia and blood sampling in the golden perch, *Macquaria ambigua.*** *Comparative Biochemistry and Physiology, A.* 103A(3):445-450
NAL Call No. QP1 C6
The major metabolic changes associated with repeated capture, aquarium transfer, anaesthesia and blood sampling were investigated in an Australian freshwater fish, the golden perch (*Macquaria ambigua*). A compounded stress response was seen after repetition of the procedure, in which the plasma glucose rose within 3 hr and amino acid concentrations rose and the serum free fatty acids concentration fell after 24 hr. Alanine was identified as an important circulating energy store in the stress response of golden perch. No change was noted in the serum protein, plasma lactate or beta -hydroxybutyrate concentrations, indicating that tissue damage and hypoxia were absent, and that degradation of free fatty acids did not produce metabolites excess to the requirements of gluconeogenesis and the tricarboxylic acid cycle.
Descriptors: freshwater fish, fish physiology, metabolism, blood, fatty acids, anaesthesia, biological stress, *Macquaria ambigua*, glucose, amino acids, lactate, beta -hydroxybutyrate, levels, sampling, capture
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Brattelid T, Smith AJ (2000) **Methods of positioning fish for surgery or other procedures out of water**. *Laboratory Animals*. 34 (4):430-433
NAL Call No. QL55 A1L3
Descriptors: fish, surgery, techniques, animal welfare

Brown LA (1987) **Recirculation anaesthesia for laboratory fish.** *Laboratory Animals.* 21(3):210-215
NAL Call No. QL55 A1L3

An economic reliable long-term recirculation anaesthesia system for laboratory fish is described. Anaesthesia of channel catfish (*Ictalurus punctatus*) was induced within 60 s and was maintained for up to 40 min using tricaine methanesulphonate; recovery occurred within 30-60 s. Various surgical procedures were performed on the fish. No deaths were recorded. All water-quality parameters tested over 19 days use of the system remained stable except for total ammonia nitrogen and, by calculation, un-ionized ammonia which increased to a maximum of 0 multiplied by 23 mg/l.
Descriptors: anesthesia, aquaria, recirculating systems, fish culture, *Ictalurus punctatus*

Bruecker P, Graham M (1993) **The effects of the anesthetic ketamine hydrochloride on oxygen consumption rates and behaviour in the fish *Heros (Cichlasoma) citrinellum* (Guenther, 1864).** *Comparative Biochemistry and Physiology, C.* 104C(1): 57-59
NAL Call No. QP901 C6
Heros citrinellum (0.106 to 0.357 kg) were injected with 30 mg/kg ketamine hydrochloride via the dorsal aorta or caudal vein. Immediate loss of balance and complete cessation of fin movement was observed post injection for one to 41 min. Ventilation was barely perceptible or ceased altogether within one minute after injection. Balance returned within 57 to 263 min after injection. Oxygen consumption rate was significantly higher during anesthesia than during control experiments, and significantly lower than during sham treatments. The safety margin of the drug was reduced at tropical temperatures versus temperate conditions. The usefulness of this drug in reducing the stress of prolonged handling, such as during transport, was not indicated. However, injectable ketamine would be useful in reducing the effects of handling larger freshwater tropical fish over shorter time periods.
Descriptors: fish physiology, anaesthesia, pharmacology, hazard assessment, temperature effects, *Heros citrinellum*, anaesthetics, ketamine hydrochloride, oxygen consumption, behaviour, Pisces

Carrasco Meza S (1983) **Immobilization of carp(*Cyprinus carpio*), catfish (*Ictalurus punctatus*) and tilapia (*Tilapia mossambica*) using xylocaine with sodium bicarbonate.** Thesis, Universidad Nacional Autonoma de Mexico (Mexico). Fac. de Med. Vet. Zootec. 33 pp.
Anesthetic solutions (xilocaine and xilocaine with sodium bicarbonate) were tested on carp (*Cyprinus carpio*), catfish (*Ictalurus punctatus*) and tilapia (*Tilapia mossambica*). The fish were immersed in the 2 solutions so that the anesthetic would penetrate the gills by diffusion. The catfish was the most susceptible (250 mg/l.) followed by the carp (350 mg/l.) and the tilapia (2000 mg/1.). The carp and catfish had a similar induction period. Tilapia showed the smallest induction period but needed the highest xilocaine concentration. Xilocaine with sodium bicarbonate reduced the induction period in carps and in some cases in catfish and tilapia. In tilapia the xilocaine concentration was reduced to 250 mg/l. and 350 mg/l. An increment in the recovery period was observed in the 3 species. The use of xilocaine with sodium bicarbonate gave more practical results.
Descriptors: anesthetics, *Cyprinus carpio, Ictalurus punctatus, Tilapia mossambica*, freshwater fish, Mexico, xylocaine

Carrasco S, Sumano H, Navarro-Fierro R (1984) **The use of lidocaine-sodium bicarbonate as anaesthetic in fish.** *Aquaculture* 41(4):395-398
NAL Call No. SH1A6

Lidocaine and lidocaine-sodium bicarbonate mixture were evaluated as anaesthetics for carp (*Cyprinus carpio*), catfish (*Ictalurus punctatus*) and tilapia (*Tilapia mossambica* = *Oreochromis mossambicus*). Although both induced anaesthesia, the mixture was more effective for induction and recovery, and was able to achieve the required time for the fish to be out of water.
Descriptors: anesthetics, lidocaine, animal breeding, aquaculture, *Cyprinus carpio, Ictalurus punctatus, Oreochromis mossambicus*, lidocaine, lidocaine-sodium bicarbonate
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Cataldi E, Di Marco P, Mandich A, Cataudella S (1998) **Serum parameters of Adriatic sturgeon *Acipenser naccarii* (Pisces: Acipenseriformes): effects of temperature and stress.** *Comparative Biochemistry and Physiology, A.* 121A(4):351-354
NAL Call No. QP1 C6
Data on the concentrations of some blood constituents of captive Adriatic sturgeon, *Acipenser naccarii*, a primitive bony fish, are reported. Serum osmolality, Na^+, K^+, Cl^-, Ca^{++}, cortisol, glucose and total protein concentrations were measured. The effects of anaesthesia, temperature, crowding and prolonged handling stress were tested on a group of 12 4-year-old sturgeons sampled repeatedly. The anaesthetic does of MS 222 (140 mg/l) induced significant osmolality elevation in the sturgeon. After exposure to colder temperature (17°C versus 25°C), cortisol and Cl^- concentrations significantly decreased. The cultured sturgeon did not seem susceptible to crowding and prolonged handling stress, since neither the serum cortisol and glucose levels nor the other blood parameters were affected by these stressors. Results are compared with the few available data on other chondrostean fish and with those on teleosts.
Descriptors: captivity, biological stress, haematology, serological studies, temperature effects, fish culture, anaesthesia, *Acipenser naccarii, Adriatic sturgeon*
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Chapman DC, Jackson UT, Hubert WA (1988) **Method for separating normal striped bass larvae from those with uninflated gas bladders.** *Progressive Fish-Culturist.* 50(3):166-169
NAL Call No. 157.5 P94
The anesthetic tricaine was used to separate normally developing larvae of striped bass (*Morone saxatilis*) from larvae with uninflated gas bladders. The procedure was most successful between 23 and 40 d posthatch, and was used to provide thousands of striped bass larvae in which the frequency of gas bladder inflation exceeded 99%. Few normally developing fish were lost due to handling mortality or inadvertent disposal with fish having uninflated gas bladders. The procedure has applications in research, production-scale fish culture, and fishery management.
Descriptors: grading, swim bladder, anaesthetics, abnormalities, *Morone saxatilis*, fish culture, normal vs. uninflated, tricaine
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Chatain B, Corrao D (1992) **A sorting method for eliminating fish larvae without functional swimbladders.** *Aquaculture.* 107(1):81-88
NAL Call No. SH1 A6
The authors describe a simple sorting method for separating cultured fish larvae with functional swimbladders from those without based on density differences. The whole population was first anaesthetized with MS 222 and then the fish were separated: fish with a functional swimbladder float and those without sink. The efficiency of the separation method

was tested at several anaesthetic doses (0.02 to 0.1 g/l) with sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus auratus*) larvae in the 6-34 mm (total length) range. The minimal sorting size was 15 mm for sea bass with an optimal anaesthetic dose of 0.07 g MS 222/l. There were not enough data to draw conclusions for sea bream. The method was satisfactory when applied in real conditions to a large (90,000) population of sea bass fry with an efficiency ratio of over 80%.
Descriptors: fish larvae, aquaculture techniques, fish culture, *Dicentrarchus labrax*, *Sparus aurata*, swim bladder, separation processes
ASFA; Copyright © 2003, FAO

Chiba A, Chichibu S (1992) **High-energy phosphate metabolism in the phenthiazamine hydrobromide anesthetized loach *Cobitis biwae*.** *Comparative Biochemistry and Physiology, C.* 102C(3):433-437
NAL Call No. QP1 C6
Changes in the metabolism of high-energy phosphates after administration of an anesthetic, phenthiazamine hydrobromide (2-amino-4-phenylthiazole; APT), were studied in the loach (*Cobitis biwae*) by in vivo ^{31}P nuclear magnetic resonance (^{31}P -NMR). Anesthetic effects appeared at about 7 min after the loach was placed in 50 ppm APT solution. Coincident increase in phosphocreatine (PCr) and a decrease in inorganic phosphate (Pi) were observed. PCr returned to the preanesthetic level when the anesthetic solution was replaced with fresh water. beta -ATP was almost unchanged during APT anesthesia.
Descriptors: fish physiology, animal metabolism, ATP, anaesthesia, *Cobitis biwae*
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Cho G, Heath D (2000) **Comparison of tricaine methanesulphonate (MS222) and clove oil anaesthesia effects on the physiology of juvenile chinook salmon *Oncorhynchus tshawytscha* (Walbaum).** *Aquaculture Research.* 31(6):537-546
NAL Call No. SH1 F8
This study investigated the feasibility of using clove oil as an alternative to tricaine methanesulphonate as a fish anaesthetic, particularly in fish stress research. The physiological stress responses of juvenile chinook salmon *Oncorhynchus tshawytscha* (Walbaum) anaesthetized with either tricaine (50 mg/L or clove oil (20 p.p.m.) were compared using unanaesthetized fish as controls. Haematocrit, serum cortisol and serum glucose concentrations, serum lysozyme activity and differential leucocyte counts were measured from blood samples collected before, during and upon recovery from anaesthesia and at specified intervals up to 72 h after recovery. Differences between the two anaesthetic groups were not significant for most of the physiological traits measured. Serum lysozyme activity of control fish, however, was significantly suppressed relative to the treated fish for 72 h after stress. Clove oil may be a safe and cost-effective alternative to tricaine without significantly affecting study results. Furthermore, clove oil may be more practical for field-based research, because a withdrawal period is unnecessary, and clove oil does not pose an environmental hazard.
Descriptors: fish culture, anaesthesia, aquaculture techniques, biological stress, fish physiology, *Oncorhynchus tshawytscha*, Chinook salmon
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Cho YJ, Cho MS, Kim SM, Choi YJ (1997) **Effect of anesthesia killing and non-bleeding on physicochemical properties of plaice *Paralichthys olivaceus* muscle at early period after death.** *Journal of the Korean Fisheries Society. Pusan. 30(4):589-594*

ISSN: 0374-8111
This study was performed to clarify the effect of anesthesia killing and non-bleeding on the physicochemical and rheologicai properties of plaice *Paralichthys olivaceus* muscle at early period after death. Live plaice was killed by 2 different methods: spiking at the brain instantly with bleeding or dipping in seawater containing anesthetic (2,000 ppm ethyl-aminobenzoate) for 10 min without bleeding. These samples were stored at 0°C and used in checking rigor-mortis, ATP breakdown, the content of ATP and its compounds, breaking strength, and lactate accumulation through storage. The rigor-mortis ATP breakdown, and lactate accumulation was faster in samples killed by spiking than in anesthesia. ATP in samples killed by anesthetic showed little breakdown until 22.5 hrs, but it was decomposed completely after 30 hrs storage. Breaking strength of samples killed by spiking at the brain instantly with bleeding decreased steadily and showed the maximum value over 10 hrs (2207.3 plus or minus 60.2 g). However, in the case of the dipping fresh flesh without bleeding in seawater containing anesthetic, the value and time reached around the maximum breaking strength were 2147.8 plus or minus 29.0 g and 13 hrs respectively, but it maintained constantly until 20 hrs passed. From these results it could be suggested that anesthesia killing and non-bleeding is more effective in maintaining firmness of fresh plaice muscle than spiking killing with bleeding at the early period after death.
Descriptors: food fish, anaesthesia, physicochemical properties, muscles, rheology, quality assurance, *Paralichthys olivaceus*, spiking, bleeding

Chung KS (1980) **Cold anaesthesia of tropical fish.** *Bulletin of the Japanese Society for Scientific Fisheries.* 46(3):391
NAL Call No. 414.9 J274
Descriptors: fish physiology, temperature tolerance, anaesthesia, tropical fish, cold anaesthesia

Clark KJ (2000) **Temperature and species comparisons of benzocaine pharmacokinetics, metabolism and physiologically based pharmacokinetic model within channel catfish, *Ictalurus punctatus*, and Yellow Perch, *Perca flavescens*.** *Dissertation Abstracts International Part B: Science and Engineering.* 60(8):3880
NAL Call No. Film S-1806
Benzocaine, a local anesthetic for mammals, has potential for use as a general anesthetic in finfish. Its pharmacokinetics, metabolism, and residue profiles were characterized to support its approval in fish. To compare interspecies and temperature differences of these parameters channel catfish were studied at 16°C, 21°C, and 26°C and yellow perch at 16°C. The feasibility of "crop grouping" was investigated with a physiologically based pharmacokinetic (PBPK) model as a means to reduce the amount of testing required for aquaculture drug registration. Plasma concentration-time profiles and residue depletion profiles of benzocaine in fish tissues and fluids were characterized after fish were administered an intraarterial constant-rate infusion of 60.5 mu mol/kg benzocaine for 30 minutes. The tissue partition coefficients for benzocaine and its main metabolites were determined by fish exposed to 18.4 mu mol/L benzocaine bath for 24 hours. Benzocaine, acetylbenzocaine, p-aminobenzoic acid, and acetyl-p-aminobenzoic acid concentrations were determined by reverse isotope dilution using HPLC and LS counting. The PBPK model was comprised of seven tissue groups connected by a parallel circulatory system, in which elimination was by liver and trunk kidney metabolism, and branchial excretion. Benzocaine disappearance from plasma conformed to a two-compartment model. Comparison between benzocaine metabolic

clearances and total body clearances implied that blood flow across the gills, not metabolism, was its primary route of elimination. By 48 hours, less than 2.1% of the dose remained in the fish, of which a small percentage was in the white muscle (edible tissue). The benzocaine blood-to-water concentration ratio was 2.17-2.80 for catfish and 5.47 for perch. The magnitude of the tissue partition coefficients suggested that tissues would not retain benzocaine if elimination were perfusion rate limited. Benzocaine was 63-76% nonsaturable bound to plasma proteins. In fact the combined low affinity, high capacity and high affinity, low capacity plasma protein binding accounted for the rapid initial elimination of benzocaine followed by a longer terminal elimination. The excellent correspondence between experimental and simulated data suggested that the PBPK model was representative of the "true" physiology of fish with regard to benzocaine exposure.
Descriptors: fish culture, anaesthesia, toxicity tests, biodegradation, fish physiology, chemical kinetics, temperature effects, bioaccumulation, *Ictalurus punctatus, Perca flavescens*, channel catfish, graceful catfish, yellow perch
ASFA; Copyright © 2003, FAO

Cooper AR, Morris S (1998) **The blood respiratory, haematological, acid-base and ionic status of the Port Jackson shark, *Heterodontus portusjacksoni*, during recovery from anaesthesia and surgery: a comparison with sampling by direct caudal puncture.** *Comparative Biochemistry and Physiology, A.* 119A(4):895-903
NAL Call No. QP1 C6
The effects of caudal cannulation on the blood physiology of the Port Jackson shark, *Heterodontus portusjacksoni*, were investigated in sharks given between 4 and 72 h to recover from surgery. Neither the PaO_2-Pv O_2 difference nor the CaO_2-CvO_2 difference of cannulated sharks fluctuated throughout the sampling period. The plasma acidosis exhibited 4 h after surgery was partially compensated after 24 h by a respiratory (hyperventilatory) alkalosis and after 72 h by a marked metabolic alkalosis. Whilst *H. portusjacksoni* exhibited some cell swelling after surgery the haematological status of cannulated sharks generally varied little throughout the recovery period. In contrast, marked changes in plasma and erythrocyte ion concentrations were indicative of increased branchial and erythrocyte ion permeability. The blood status of *H. portusjacksoni* given 72 h to recover from surgery was also compared with sharks sampled by caudal puncture. The respiratory and acid-base status of sharks sampled by caudal puncture was comparable to that of cannulated sharks. In contrast, the plasma ion concentrations of the cannulated sharks were markedly elevated and the erythrocyte ion concentrations concomitantly reduced when compared with punctured sharks. The apparent increase in the water and ion permeability of cannulated sharks was reflected by the reduced [Hb] and mean cell haemoglobin concentrations (MCHC). Blood sampling by caudal puncture appeared to reduce the haematological and ionic perturbations that resulted from surgery and thus provided a less invasive and reliable method for obtaining samples from 'non-disturbed' elasmobranchs.
Descriptors: fish physiology, anaesthesia, haematology, pH effects, biological stress, *Heterodontus portusjacksoni*, Port Jackson shark
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Cubero L, Molinero A (1997) **Handling, confinement and anaesthetic exposure induces changes in the blood and tissue immune characteristics of gilthead sea bream.** *Diseases of Aquatic Organisms.* 31(2):89-94
ISSN: 0177-5103
The gilthead sea bream *Sparus aurata* is a species of great interest for aquaculture, and in the

last few years its culture has increased in the Mediterranean. This study was carried out to elucidate the mechanisms underlying the immunological response of fish after handling and confinement, procedures commonly associated with fish transport, as well as to determine the putative protective role of an anaesthetic (tricaine methanosulphonate) during confinement. Handling produces changes not only in circulating blood cells but also in the immunological cell populations of the thymus, spleen and pronephros. A stronger response in immunological cells was obtained when handling was followed by confinement. Circulating white blood cells returned to normal approximately 48 h after the onset of stress, whereas immunological tissue cells recovered later. The presence of an anaesthetic partially prevented the circulatory response, suggesting that the immunological response was less and supporting therefore the belief that the anaesthetic plays a protective role. However, it worsened the effect of handling plus confinement on haematopoietic organs, indicating that examining immunological cells in circulation only can lead to a false conclusion. Our results suggest that fish are not completely recovered until at least 144 h (6 d) after handling and transport, when cellular recovery in immunological organs occurs.
Descriptors: fish culture, fish handling, anaesthetics, fish physiology, haematology, leukocytes, immunity, tissues, *Sparus aurata*
ASFA; Copyright © 2003, FAO

Culloty SC, Mulcahy MF (1992) **An evaluation of anaesthetics for *Ostrea edulis* (L.).** Pamaq IV: Fourth International Colloquium On Pathology In Marine Aquaculture. *Aquaculture.* 107(2-3):249-252
NAL Call No. SH1 A6
An effective anaesthetic for oysters was sought, in order to allow for experimental manipulation of live oysters (*Ostrea edulis*) with minimal stress. A range of anaesthetic commonly used with gastropods and cephalopods, as well as a fish anaesthetic, were tested. Most of the chemicals tested were found to be unsuitable for oysters. Magnesium chloride was the most successful agent, inducing anaesthesia quickly, allowing rapid recovery with minimal stress and mortality.
Descriptors: anaesthesia, drugs, *Ostrea edulis*, oyster culture, pharmacology
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Davidson GW, Davie PS, Young G, Fowler RT (2000) **Physiological responses of rainbow trout *Oncorhynchus mykiss* to crowding and anesthesia with AQUI-S super(TM).** *Journal of the World Aquaculture Society.* 31(1):105-114
NAL Call No. SH138 W62
Following exposure to the anesthetic AQUI-S super(TM), plasma cortisol concentration in immature rainbow trout was measured as (mean) 293 plus or minus 48 ng/mL, which was significantly ($P > 0.05$) higher than the mean concentration in resting fish. Cortisol concentrations remained significantly ($P > 0.05$) elevated for at least 24 h after treatment. This was accompanied by a significant increase and decrease in hematocrit and plasma potassium, respectively. These perturbations continued for at least 48 h following recovery from anesthesia. Plasma concentrations of total protein and sodium remained unchanged following anesthesia with AQUI-S super(TM). Crowding stress is commonly encountered by fish during manipulation in aquaculture situations. Anesthetising fish prior to, and during, manipulation may reduce the associated stress. Changes in cortisol values resulting from crowding (30 min; 0.1 kg/L) during anesthesia with AQUI-S super(TM) were not appreciably different from those in fish crowded without anesthesia. Thus, anesthesia with AQUI-S super(TM) at the recommended dose of 17 mg/L did not appear to be effective for

alleviating the stress of crowding under the conditions of our experiments.
Descriptors: fish culture, anaesthesia, fish physiology, biological stress, hormones, *Oncorhynchus mykiss*, rainbow trout
ASFA; Copyright © 2003, FAO

de Carvalho Gomes L (2001) **Effect of temperature on the safest level of benzocaine as an anesthetic for juveniles of *Colossoma macropomum*.** *Aquaculture 2001: Book of Abstracts.* p. 251.
Benzocaine is widely used and less expensive anesthetic for fish in Brazil. Therefore, the present investigation is to define an ideal dose of benzocaine for juveniles of *Colossoma macropomum (tambaqui)*. Fishes were exposed to various doses i.e. 50, 100, 150, 200 and 250 mg/L at different temperatures i.e. 24°C, 27°C, and 30°C and the behavioral events were taken in to consideration. In all acclimation temperatures 100 mg/L was the concentration considered ideal for quickly inducing total mobilization, attained fast recovery and safe. This concentration influence the inducing time and minimize it as the temperature rises. In this concentration, fishes may safely be exposed even for 20 minutes. Neither of the concentrations exhibited mortality while exposed at different temperatures. In contrast to other studies benzocaine is an anesthetic with a high safety margins. The fishes acclimatized at 27°C treated with 100 mg/L and exposed for 20 minutes the recovery time exposed was significantly higher than the fish exposed to anesthetic for 10 min. There were no significant differences in the recovery time at other temperatures. No mortality could be observed after 24 h of exposure for 10 or 20 min in all temperatures. Behavioral events (minutes) of *C. macropumum* juveniles exposed to Benzocaine.
Descriptors: temperature effects, anaesthetics, aquaculture techniques, *Colossoma macropomum*, Brazil, benzocaine, juveniles
ASFA; Copyright © 2003, FAO

de Carvalho Gomes L, Roubach R, Araujo-Lima CARM (2002) **Effect of density on survival and water quality for transporting *Colossoma macropomum* juveniles with CO_2 as an anesthetic.** *Aquaculture 2001: Book of Abstracts.* p. 252
Transportation of live fish is an essential procedure for fish culture and management. Previous studies demonstrated that the mortality of *Colossoma macropomum* juveniles decreased with the addition of CO_2 (50 mg/L) as an anesthetic during transportation. The aim of this paper is to verify the effect of high density on mortality and water quality during transportation with CO_2. Juveniles were stocked in the bags in different density (fishes/L) i.e. 30, 60, 80 and 100. CO_2 was added to the water by bubbling the gas. These bags were maintained for 24 h and mortality and physico-chemical parameters of water were monitored. A significant correlation between density and mortality was observed, increased the mortality with density. Transportation of *C. macropomum* without anesthetics showed a mortality rate of 10-30%. Transportation with the addition of CO_2 exhibited a negligible mortality at 30 fishes/L. However, 60 and 100% of mortality were recorded at 80 and 100 fishes/L. Concentrations of CO_2 and O_2 in the water with high densities were respectively high and low with the lethal range for many fish species. However no significant relation could be noticed between these parameters and the mortality rate. In spite of correlation not being observed in isolated parameters the combination of the high CO_2 and low O_2 concentrations is the most probable explanation for the mortality of fish transported in high densities. Therefore, the addition of CO_2 as an anesthetic in low densities (30 fishes/L) for long transportation is recommended.
Descriptors: water quality, aquaculture techniques, anaesthetics, carbon dioxide, dissolved

oxygen, transportation, population density, survival, mortality causes, *Colossoma macropomum,* Brazil, juveniles
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de M C Gontijo AM, Barreto RE, Volpato GL, Reyes VAV, Salvadori DMF (2001) **Why not anesthetize fish? In vivo and ex vivo evidence of no interference of benzocaine in the comet assay.** *Mutation Research.* 483(Supplement 1):S171
NAL Call No. QH431.M8
Descriptors: animal welfare, biomonitoring, fish, benzocaine, local anesthetic, alkaline comet assay, methods, blood

Deacon N, White H, Hecht T (1997) **Isolation of the effective concentration of 2-phenoxyethanol for anaesthesia in the spotted grunter, *Pomadasys commersonnii*, and its effect on growth.** *Aquarium Sciences and Conservation.* 1(1):19-27
ISSN: 1357-5325
2-Phenoxyethanol is a highly suitable anaesthetic for use with fish. This paper describes the isolation of an effective concentration of this anaesthetic for use with the spotted grunter, *Pomadasys commersonnii*. Routine anaesthesia with 2-phenoxyethanol was found to have no significant effect on the growth of this fish.
Descriptors: aquaculture techniques, fish culture, marine aquaculture, anaesthetics, *Pomadasys commersonnii*, growth
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Derozier C (1989) **Phenoxy-2 Ethanol anaesthesia in fish.** *Thesis, Ecole Nationale Veterinaire, Nantes (France).* 100 pp.
The author studies the anaesthetic effects of phenoxy-2 ethanol in comparison with other anaesthetics utilized in aquaculture. Phenoxy-2 ethanol does not offer great advantages except for better safety, but is less expensive. Further studies must determine whether the flesh of anaesthetized fish retain phenoxyl-2 ethanol residues liable to be noxious to human health.
Descriptors: anaesthesia, toxicants, toxicity tests, aquaculture techniques, fish culture, Pisces, phenoxy-2 ethanol
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Dick G (1973) **Some observations on the use of MS 222 Sandoz trial I.** *Scientific Report, Research and Development Department, White Fish Authority (UK).* pp. 1-7
(1) The fish are much easier caught and handled when tranquillised, they are less active and are liable to do themselves less damage in this state. (2) The fish would be less liable to stress by disturbances such as might be incurred in transportation, bumping etc., if the fish were tranquillised (3) The use of MS222 Sandoz at low concentrations of 1:75,000 and 1:100,000 would be sufficient to slow the fish down to enable catching and also to keep them tranquil during transportation for a duration of 48 hours. (4) There seems little observable difference in the effect of 1:75,000 and 1:100, 000 on the fish over 48 hours, 1:100, 000 would be cheaper. (5) To use high concentrations of 1:10,000 and 1:30,000 would be dangerous if dealing with large numbers of fish. The operation would have to be carried out swiftly as prolonged immersion has been found to be lethal after 45 minute and 9 hours respectively. At these concentration the fish would become completely anaesthetised. (6) It is felt that at a concentration of 1:50,000 over 48 hours may well have produced unmeasurable internal

damage. After 22 hours, the fish seem to be fighting against the anaesthetic and kept this up for the duration of time.
Descriptors: MS222 Sandoz, anesthesia, stress, concentration, dosage

Durve VS (1975) **Anaesthetics in the transport of mullet seed.** *Aquaculture.* 5(1):53-63

The author reports the results of an investigation on the use of anaesthetics in the transportation of live mullet seed. Experiments were carried out on 13 anaesthetics to ascertain the dosages suitable for transportation of live fish. These doses were further tested for the tolerance of mullet fry and fingerlings for a maximum period of 2 hr. Metabolism experiments were performed to find out the degree of decrease in the metabolism in terms of active O_2 consumption. Finally, results are given of the trial consignment despatched by rail for a transport period of 20 hr. The results indicate that out of the 13 anaesthetics tried, 7 were suitable for transportation of live fish. The relative merits and demerits of these anaesthetics are further discussed.
Descriptors: anaesthesia, fish culture, Mugilidae

Edwards S, Burke C, Hindrum S, Johns D (2000) **Recovery and growth effects of anaesthetic and mechanical removal on greenlip (*Haliotis laevigata*) and blacklip (*Haliotis rubra*) abalone.** *Journal of Shellfish Research.* 19(1):510

Haliotis laevigata (39.7 plus or minus 0.2 mm, 8.2 plus or minus 0.1 g) and *Halitos rubra* (41.9 plus or minus 0.1 mm, 11.3 plus or minus 0.1 g) were acclimatised to conditions over 3-5 weeks (80 animals per 80 cm diameter fibreglass tank, flow-through sand-filtered seawater 17°C, artificial diet adlib). Animals were then removed from the tanks using ethanol (3%), 2-phenoxyethanol (1 mL/L), benzocaine (100 ppm), clove oil (0.5-1.5 mL/L) and mechanical removal (metal spatula), measured, and returned to clean water for a further six weeks. At treatment, a sample of the animals was transferred to a multichannel flow-through respirometer for analysis of oxygen uptake, which lasted at least 3 days. All treatments were duplicated for both species. One set (control) remained undisturbed from the beginning of the acclimation period to the end of the trial. Additional respirometry trials were conducted on the same cohorts for KCl (10 g/L), Aqui-S (50 ppm) and Tabasco (10 ml/L) that didn't fit in the growth trial. Apart from first hour suppression (ethanol) or stimulation (clove oil & Aqui-S) of oxygen uptake, most agents showed shifted normal patterns of oxygen uptake settling to a normal value (similar to 55 mg O_2 times kg/h) over 3-5 days. Increases in oxygen uptake were seen in first day averages for clove oil (156%), Aqui-S (154%) and KCl (127%). Mechanical removal gave first day suppression (50%) of oxygen usage, returning to normal with the evening activity cycle. Tabasco treated animals took longest to recover from a suppression of oxygen uptake. Benzocaine and KCl treated animals recovered most rapidly. There was no apparent recovery from clove oil in the time period studied. Growth trials showed healthy growth rates for control *H. laevigata* (116 plus or minus 3 mu m, 78 plus or minus 4 mg per day) and all treatments indicated a suppression of growth rate as a result of removal from the tanks (48-83 mu m, 19-70 mg per day). For *H. rubra*, control growth rates were much lower (24 plus or minus 1 mu m/day) and weight gain was erratic (34 plus or minus 10 mg/day). Nonetheless, lower growth rates (length 1.4-12.1 mu m/day) were obtained for all treatments, while all but one treatment also had lower weight gain than control animals. Animals subjected to clove oil had the lowest weight gain and this was the

only treatment that resulted in significant mortalities.
Descriptors: fish diseases, mollusc culture, anaesthesia, disease control, *Haliotis laevigata, Haliotis rubra*
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Erdmann MV (1999) **Clove oil: an 'eco-friendly' alternative to cyanide use in the live reef fish industry?** *Live Reef Fish Information Buletin.* 5:4-7
ISSN: 1026-2040
The use of clove oil in aquarium trade has been a new idea in Indonesia. The author reviews the use and anaesthetic effects of clove oil. He explains the possible use in live reef fish trade. The use of cyanide is fishing is very damaging to the marine environment, hence a possible alternative - clove oil as anaesthetic for wild capture, handling and transport of live fish.
Descriptors: ornamental fish, stupefying methods, fish poisoning, trade, cyanides, live storage, transportation, aquaria, clove oil
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Etienne T (1989) **Fish anaesthesia.** *Thesis, Ecole Nationale Veterinaire, Nantes (France).* 71 pp
After a brief review of physiological and anatomical particularities of fish, the author presents the various methods of chemical and physical anaesthesia: (anaesthetics, electronarcosis, hypothermia).
Descriptors: anaesthesia, aquaculture techniques, fish culture, fish physiology, literature reviews, Pisces
ASFA; Copyright © 2003, FAO

Falls WW, Vermeer GK, Dennis CW (1988) **Evaluation of etomidate as an anesthetic for red drum, *Sciaenops ocellatus*.** Red Drum Aquaculture. Proceedings Of A Symposium On The Culture Of Red Drum And Other Warm Water Fishes.*Contributions in Marine Science.* 30 (suppl):37-42
NAL Call No. 442.9 T31
Etomidate was evaluated as an anesthetic in fourteen female and seven male red drum. Fish were anesthetized every 2 or 3 weeks with etomidate dosage levels of 0.8, 1.6, or 8.0 mg/L during a pre-spawning conditioning regime. The 8 mg/L dosage was found to be too high as fish reached stage 4 anesthesia (loss of reflex activity) in less than 30 sec. At a 0.8 mg/L dosage, mean induction and recovery times were excessively long. Further trials at these 2 dosages were discontinued. Between the induction and recovery periods, fish were maintained in a respirator at a 0.4 mg etomidate/L dosage for a mean time of 17.47 min. An induction dose of 1.6 mg/L and a respirator maintenance dose of 0.4 mg/L appeared suitable for routine fish handling.
Descriptors: anaesthetics, fish handling, *Sciaenops ocellatus*, aquaculture techniques
ASFA; Copyright © 2003, FAO

Farwell CJ (1978) **The use of the fish anesthetic quinaldine at Scripps aquarium-museum.** *Annual Proceedings of the American Association of Zoologists and Parks Aquarists.* 61-71
The use of an anaesthetic such as quinaldine when removing fish from a display tank not only simplifies the job, but helps in lowering the chances of damaging and stressing the fish that can lead to future health problems. The amount of quinaldine needed is determined by tank volume and the final concentration required and the stages of anaesthesia observed in the fish are first irritation or hyperactivity, which is quickly followed by a loss and then a

total loss of equilibrium. At this point a high respiratory rate that is weak and interrupted by gasps is apparent. The fish are relatively still and can be handled without struggling. The fish recover quickly from the effects.
Descriptors: aquaria, ornamental fish, anaesthetics, fish handling
ASFA; Copyright © 2003, FAO

Ferck BJ, Jameson JD, Ramadhas V (1996) **Carbonic acid as an anaesthetic in the transport of fishes.** *Water quality issues in aquaculture. Proceedings of the National Seminar on Water Quality Issues in Aquaculture Systems, Dec. 18 and 19, 1996.* pp. 103-108.
Fish seed and broodfish usable in fish farming or for sale need to be transported in live condition which may involve considerable time and distance. In this connection, their packing with anaesthetics is indispensable. The present investigation was undertaken to determine the safer limit of carbonic acid as an aneasthetic for the the transport of fry of 4 species of ornamental fishes viz. *Gymnocorymbus ternetzi*, *Mollienesia latipinna*, *Carassius auratus* and *Xiphophorus helleri*. The fish fry of these species were sedated with carbonic acid in 50, 100, 150 and 200 ppm concentrations with and without oxygen. Periods for indiction, recovery and death were recorded simultaneously for all the species. An extremely low induction time of 13.3 seconds at 200 ppm carbonic acid was recorded for *G. ternetzi* and *M. latipinna* and a high recovery time of 187-195 seconds of *X. helleri* and *M. latipinna*, *C. auratus* and *G. ternetzi* exhibited 80-100% survival in all the concentration of carbonic acid. The technology of packing commercially important fishes using the above anaesthetic is also discussed in detail.
Descriptors: ornamental fish, anaesthetics, carbonic acid, *Gymnocorymbus ternetzi, Mollienesia latipinna, Carassius auratus, Xiphophorus helleri*, transportation, oxygen consumption, survival, green swordtail
ASFA; Copyright © 2003, FAO

Ferreira JT, Schoonbee HJ, Smit GL (1984) **The anaesthetic potency of benzocaine-hydrochloride in three freshwater fish species.** *South African journal of zoology = Suid-Afrikaanse tydskrif vir dierkunde.* 19(1):46-50
NAL Call No. QL336 Z6
Anaesthesia was induced in the common carp, *Cyprinus carpio*, tilapia, *Oreochromis mossambicus* and rainbow trout, *Salmo gairdneri*, at concentrations of 25; 50; 75 and 100 mg/l of benzocaine-hydrochloride as well as neutralized benzocaine-hydrochloride at water temperatures of 15^{o}; 20^{o} and 25^{o}. The results obtained indicated intra- and interspecific differences in the susceptibility of fish to anaesthesia due to metabolic, chemoreceptive and temperature tolerance differences in and amongst the three species.
Descriptors: anesthetics, performance assessment, *Cyprinus carpio, Oreochromis mossambicus, Salmo gairdneri*, benzocaine-hydrochloride
ASFA; Copyright © 2003, FAO

Ferreira JT, Schoonbee HJ, Smit GL (1984) **The use of benzocaine-hydrochloride as an aid in the transport of fish.** *Aquaculture.* 42(2):169-174
NAL Call No. SHI A6
The potential of benzocaine-hydrochloride as an aid in the transport of fish was investigated. When used at a concentration of 25 mg/l the anaesthetic caused a reduction in the excretion of ammonia and carbon dioxide by the fish, while, as a result of the reduced activities of the fish, the pH and alkalinity values of the transport water remained fairly constant. When benzocaine-hydrochloride was not used, the activities of the fish produced a significant

deterioration in water quality evidenced by the accumulation of ammonia and carbon dioxide.
Descriptors: fish handling, transportation, anesthetics, aquaculture, ethyl aminobenzoate hydrochloride, Pisces, benzocaine-hydrochloride
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Ferreira JT, Schoonbee HJ, Smit L (1984) **The uptake of the anaesthetic benzocaine hydrochloride by the gills and the skin of three freshwater fish species.** *Journal of Fish Biology.* 25(1):35-41
NAL Call No. QL614 J68
The uptake of benzocaine hydrochloride and neutralized benzocaine hydrochloride by the skin and the gills of *Cyprinus carpio*, *Oreochromis mossambicus* and *Salmo gairdneri* were studied. The differences observed can mainly be ascribed to degree of ionization and the lipid solubility of the anaesthetic.
Descriptors: anesthetics, gills, skin, pharmacology, *Cyprinus carpio, Oreochromis mossambicus, Salmo gairdneri*, benzocaine hydrochloride
ASFA; Copyright © 2003, FAO

Ferreira JT, Smit GL, Schoonbee HJ (1981) **Haematological evaluation of the anaesthetic benzocaine hydrochloride in the freshwater fish *Cyprinus carpio* L.** *Journal of Fish Biology.* 18(3):291-297
NAL Call No. QL614 J68
The effects of anaesthetization with different concentrations of benzocaine hydrochloride (BH) and neutralized benzocaine hydrochloride (NBH) were studied on the haematology of *C. carpio*. Due to its acidic nature and resultant effects on aquarium water, BH produces haemoconcentration effects with disturbances in acid-base function. The use of NBH, whereby water quality effects were drastically reduced, improved the general haematological profile. In contrast, haemodilution resulted when blood was sampled without the use of an anaesthetic agent.
Descriptors: anaesthetics, haematology, *Cyprinus carpio*, Cyprinidae, Pisces
ASFA; Copyright © 2003, FAO

Ferreira JT, Smit GL, Schoonbee HJ, Holzapfel CW (1979) **Comparison of anesthetic potency of benzocaine hydrochloride and MS-222 in two freshwater fish species.** *Progressive Fish Culturist.* 41(3):161-163
NAL Call No. 157.5 P94
The hydrochloride of ethyl p-aminobenzoate was synthesized and its anesthetic potency compared with that of MS-222 at concentrations of 50, 80, and 100 mg/l. The free compound of these agents in fish blood was also determined. The results indicate that benzocaine hydrochloride is a more effective anesthetic than MS-222 at the concentrations applied. Benzocaine hydrochloride is not registered for fishery use in the United States.
Descriptors: anaesthetics, freshwater fish, *Cyprinus carpio, Sarotherodon mossambicus*, Cyprinidae, Pisces, Cichlidae
ASFA; Copyright © 2003, FAO

Gerwick L, Demers NE, Bayne CJ (1999) **Modulation of stress hormones in rainbow trout by means of anesthesia, sensory deprivation and receptor blockade.** *Comparative Biochemistry and Physiology, A.* 124A(3):329-334
NAL Call No. QP1 C6

Sympathetic activation leading to increased levels of blood catecholamines, and stimulation of the hypothalamic-pituitary-inter-renal axis leading to increased cortisol, are difficult to avoid when handling animals. Yet, in research on effects of acute stress, elicitation of such responses must be minimized in the control groups. The work examines means to achieve a minimally disturbed state in rainbow trout (*Oncorhynchus mykiss*). Level of arousal was determined by adrenaline and cortisol concentrations in plasma, and by the spleen:somatic index. Fish were prepared for bleeding by rapid capture and concussion, by infusion of anesthetic into the undisturbed home tank, by confinement in black boxes, or by being fed alpha - and beta -receptor antagonists. Even when done quickly, netting and concussion yielded fish with ca. 200-pmol adrenaline/ml plasma. Cortisol was elevated (to > 10 ng/ml) within 30 s of stress initiation. Surreptitious infusion of anesthetic (2-phenoxyethanol, PE) into tanks yielded fish with lower adrenaline levels (means 19.34 and 19.58 pmols/ml in home tank and black boxes, respectively). Among fish given phentolamine and propranolol, spleen:somatic indices and plasma adrenaline were higher than in diet controls, whether undisturbed or stressed, indicative of successful receptor blockade. Since careful infusion of 2-PE yielded the lowest adrenaline levels, and requires no special apparatus, it is the method of choice for obtaining minimally stressed fish.
Descriptors: biological stress, fish handling, hormones, anaesthetics, therapy, fish culture, drugs, serological studies, endocrinology, aquaculture techniques, anaesthesia, *Oncorhynchus mykiss*, rainbow trout

Ghion F (1975) **First promising results in the use of Fluothane as general anesthetic for fish.** *Italian Review of Fish-CultureaAnd Fish Pathology.* 10(4):111-112

Fluothane (2-bromo-2-chloro-1, 1, 1, -trifluoro-ethane) a general anesthetic for humans, has been tested on euryhaline fish (*Dicentrarchus labrax*, *Sparus auratus*, *Mugil cephalus*) with promising results. The anaesthetic is bubbled into the water mixed with air and its delivery is stopped when fish start to swim erratically. Narcosis was always attained within 10 min from the beginning of anaesthesia. Fish recovery rapidly occurred when pure air was bubbled into the aquarium. Fish were safely exposed to the anaesthetic for times ranging up to 8 h. Gross evaluation of Fluothane concs inducing immediate narcosis gave values {approx} 40 ppm.
Descriptors: anaesthesia, *Dicentrarchus labrax, Sparus aurata, Mugil cephalus*

Gilderhus PA (1990) **Benzocaine as a fish anesthetic: Efficacy and safety for spawning-phase salmon.** *Progressive Fish Culturist.* 52(3):189-191

The anesthetic benzocaine was tested for efficacy and safety for spawning-phase chinook salmon (*Oncorhynchus tshawytscha*) and Atlantic salmon (*Salmo salar*) at federal fish hatcheries. Benzocaine concentrations of 25-30 mg/L anesthetized most fish in less than 3.5 min, and most fish recovered in less than 10 min after 15 min of exposure. Safety margins were narrow; both species tolerated 30 mg/L for about 20 min, but 25 min of exposure caused deaths. For 15-min exposures, concentrations of 35 mg/L for chinook salmon and 40 mg/L for Atlantic salmon were lethal.
Descriptors: fish culture, hatcheries, anaesthetics, toxicity tolerance, *Oncorhynchus tshawytscha*, *Salmo salar*, spawning, benzocaine

Gilderhus PA, Lemm CA, Woods LC III (1991) **Benzocaine as an anesthetic for striped bass.** *Progressive Fish Culturist.* 53(2):105-107
NAL Call No. 157.5 P94
Benzocaine was tested as an anesthetic on juvenile and mature adult striped bass (*Morone saxatilis*). Concentrations of 55 mg/L at 22°C to 80 mg/L at 11°C effectively anesthetized fish in about 3 min. Recovery was more rapid as temperature increased. Fish survived concentrations of twice the effective concentration and exposure times up to 60 min at the effective concentration. Striped bass required higher concentrations for anesthetization than had been previously demonstrated for salmonid fishes, but safety margins for both concentration and exposure time were wider than for the salmonids.
Descriptors: anaesthetics, efficiency, recovery, temperature effects, *Morone saxatilis*, fish culture, benzocaine
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Gingerich WH, Drottar KR (1989) **Plasma catecholamine concentrations in rainbow trout (*Salmo gairdneri*) at rest and after anesthesia and surgery.** *General and Comparative Endocrinology.* 73(3):390-397
NAL Call No. 444.8 G28
The effects of surgery and anesthesia on concentrations of plasma epinephrine (E), norepinephrine (NE), and dopamine (DA) were investigated in rainbow trout (*Salmo gairdneri*) fitted with dorsal aorta cannulae. Baseline catecholamines (CA) concentrations, established in resting rainbow trout, were 1.55 plus or minus 0.90 rho mol/ml (X plus or minus SD) for E, 2.07 plus or minus 1.26 for NE, and 1.33 plus or minus 0.87 for DA. After surgery, plasma concentrations of all CAs fell rapidly but values were still higher than baseline 6 hr after surgery, then were near baseline at 24 and 48 hr after surgery. Plasma E and NE concentrations in the fish during early anesthesia (1.14 plus or minus 0.14 min) were not significantly different from preanesthesia values.
Descriptors: blood, hormones, anaesthesia, biological stress, steroids, fish physiology, *Salmo gairdneri*, serological studies
ASFA; Copyright © 2003, FAO

Gleadall IG (1991) **Comparison of anaesthetics for octopuses.** *Bulletin of Marine Science.* 49(1-2):663
ISSN: 0007-4977
This study, comparing eleven different immersion anaesthetics for octopuses, was initiated in view of the failure of conventional ethanol or urethane anaesthesia of octopuses at lower temperatures (< 12°C), 2) a requirement for better fine control over anaesthesia, and, a reluctance to use magnesium chloride anaesthesis for certain experiments. A standard procedure was adopted to compare the three well established anaesthetics (ethanol, urethane, magnesium chloride) with several well known fish anaesthetics (tricaine, metomidate, propoxate) and those commonly employed with invertebrates (chloral hydrate, chloretone, menthol, nicotine sulphate, phenoxetol). Also, two methods of anaesthesia by cooling are briefly compared. The species used were mostly *Octopus vulgaris* and *O. fang-siao* (but also included *O. dofleini* and *Octopus sp.*, with doses carefully adjusted for body weight; three different doses were used within the recommended range for each anaesthetic. Most "invertebrate" and "fish" anaesthetics either failed to anaesthetize the octopuses (at low doses) or they proved toxic or fatal (at higher doses), with no intermediate anaesthetic effect. Effective anaesthetics are used for different types of experimentary, but the search continues for a more finely controllable, all-round anaesthetic for octopuses.

Descriptors: temperature effects, Octopoda, comparative studies, anaesthetics
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Gomes LC, Chippari-Gomes AR, Lopes NP, Roubach R, Araujo-Lima CARM (2001) **Efficacy of Benzocaine as an Anesthetic in Juvenile Tambaqui *Colossoma macropomum*.** *Journal of the World Aquaculture Society.* 32(4):426-431
NAL Call No. SH138 W62
The present study investigated the use of benzocaine as an anesthetic for juvenile *Colossoma macropomum (tambaqui)*. In the first experiment, fish were exposed to various doses of benzocaine for 10 min at 24°C. The second experiment examined the effects of duration of exposure to 100 mg/L of benzocaine. In the third experiment, fish were exposed to 100 mg/L at temperatures of 24°C, 27°C, and 30°C. Benzocaine concentrations of 100-150 mg/L were considered ideal for quickly inducing total immobilization and fast recovery. Fish exposed to 350 mg/L benzocaine exhibited 30% mortality. No changes in hematocrit were recorded in fish exposed to different concentrations of benzocaine. Plasma glucose increased significantly when fish were exposed to benzocaine concentrations greater than 200 mg/L. Recovery time after a 30-min exposure to 100 mg/L benzocaine was significantly greater than after an exposure for 10 and 20 min. No mortality was observed 96 h after exposure to 100 mg/L benzocaine for 10, 20, and 30 min. Dosages in the 100-150 mg/L range were effective for periods of up to 20 min of anesthesia. There was no effect of temperature on the time required for fish to lose equilibrium. However, recovery was significantly faster for fish anesthetized at 30°C. Benzocaine is an effective anesthetic agent for tambaqui juveniles, providing rapid immobilization and rapid recovery. Benzocaine is also less expensive than other available anesthetic compounds.
Descriptors: juveniles, experimental research, costs, anaesthetics, *Colossoma macropomum*, benzocaine
ASFA; Copyright © 2003, FAO

Graham MS, Iwama GK (1990) **The physiologic effects of the anesthetic ketamine hydrochloride on two salmonid species.** *Aquaculture* 90(3-4):323-331
NAL Call No. SH1A6
Adult coho salmon (*Oncorhynchus kisutch*) and subadult rainbow trout (*Oncorhynchus mykiss*) were used in experiments with the anesthetic ketamine hydrochloride. The drug (30 mg/kg) was injected into the dorsal aorta through an indwelling cannula. Intravascular administration of ketamine caused an immediate cessation of ventilation in both species for 10 s to 300 s and a loss of balance. Ventilation rate recovered to pre-anesthesia values within 1-2 h and arterial oxygen values were at pre-anesthesia levels by 3-24 h. Anesthesia caused a significant acidosis in both species. The blood pH and plasma CO_2 values had returned to pre-anesthesia levels by 4-24 h and 0.5-2 h, respectively. For adult salmon, five of seven animals were unresponsive to tail grabbing at 4 h while with juvenile trout, three of five fish were fully responsive to touch at 1 to 2 h. This difference in duration of anesthesia was likely size-related. The applications of this injectable anesthetic for commercial fish use, mainly in the transport of animals, are suggested, but its use with food fish has not been assessed.
Descriptors: anaesthetics, aquaculture techniques, cultured organisms, transport, *Oncorhynchus mykiss, Oncorhynchus kisutch*, ketamine hydrochloride
ASFA; Copyright © 2003, FAO

Guidobono F, Netti C, Sibilia V, Villa I, Zamboni A, Pecile A (1986) **Eel calcitonin binding site distribution and antinociceptive activity in rats.** *Peptides* 7(2):315-322
NAL Call No. QP552 P4P45
The distribution of binding sites for (^{125}I)-eel-calcitonin (ECT) to rat central nervous system, studied by an autoradiographic technique, showed concentrations of binding in the diencephalon, the brain stem and the spinal cord. Large accumulations of grains were seen in the hypothalamus, the amygdala, in the fasciculus medialis prosencephali, in the fasciculus longitudinalis medialis, in the ventrolateral part of the periventricular gray matter, in the lemniscus medialis and in the raphe nuclei. In the spinal cord, grains were scattered throughout the dorsal horns. Binding of the ligand was displaced equally by cold ECT and by salmon CT(sCT), indicating that both peptides bind to the same receptors. The administration of ECT into the brain ventricles of rats dose-dependently induced a significant and long-lasting enhancement of hot-plate latencies comparable with that obtained with sCT. The antinociceptive activity induced by ECT is compatible with the topographical distribution of binding sites for the peptide and is a further indication that fish CTs are active in the mammalian brain.
Descriptors: calcitonin, central nervous system, intracerebroventricular administration, analgesia, fish physiology, peptides, brain, Anguilla, binding, autoradiography, distribution, rats, neurophysiology
ASFA; Copyright © 2003, FAO

Guo FC, Teo LH, Chen TW (1995) **Effects of anaesthetics on the oxygen consumption rates of platyfish *Xiphophorus maculatus* (Guenther).** *Aquaculture Research.* 26(12):887-894
NAL Call No. SH1 F8
This study was carried out to find out the effects of anaesthetics, 2-phenoxyethanol, quinaldine sulphate, MS-222 and metomidate, at various dosages, on the oxygen consumption rates of two size groups of platyfish, *Xiphophorus maculatus* (Guenther) at three temperatures. The results show that the oxygen consumption by the platyfish of both size groups was temperature dependent, being higher at higher temperature, but not size dependent. The effects of anaesthetics on the oxygen consumption rates of platyfish were dosage dependent and temperature dependent, especially for 2-phenoxyethanol, the effect always being significantly greater at lower temperature. Small and large fish did not show much difference in their responses to anaesthetic treatments. However, with 2-phenoxyethanol, the effect on the large platyfish was always better than on the small ones at 20-25°C. At 220-440 ppm and at 20°C, 2-phenoxyethanol was more effective than the other anaesthetics in suppressing oxygen consumption by the platyfish.
Descriptors: anaesthetics, temperature effects, *Xiphophorus maculatus*, oxygen consumption, ornamental fish, aquaria
ASFA; Copyright © 2003, FAO

Guo FC, Teo L-H, Chen TW (1995) **Effects of anaesthetics on the water parameters in a simulated transport experiment of platyfish, *Xiphophorus maculatus* (Guenther).** *Aquaculture Research.* 26(4):265-271
NAL Call No. SH1 F8
An experiment was carried out to study the effects of anaesthetics (2-phenoxyethanol, quinaldine sulphate, metomedate and MS-222) on water parameters during simulated air transport of platyfish, *Xiphophorus maculatus*. The platyfish were put in sealed plastic bags, one-quarter full of water, to which a required amount of anaesthetic was added. The rest of the bag was filled with oxygen. The water in the bag was tested for pH, total ammonia and

carbon dioxide at intervals of 4 and 8 h for a period of 48 h. Mortality rates within this period and the post-packaging period were also noted. It was found that 2-phenoxyethanol was most effective, followed by quinaldine sulphate, in decreasing the excretion of metabolic wastes by the fish. Metomidate had no effect in the control of waste production. MS-222 reduced ammonia excretion but not carbon dioxide. None of the anaesthetics used had any effect on the pH of the water.
Descriptors: *Xiphophorus maculatus*, oxygen consumption, anaesthetics, fish physiology, excretion

Hanawa M, Harris L, Graham M, Farrell AP, Bendell-Young LI (1988) **Effects of cyanide exposure on Dascyllus aruanus, a tropical marine fish species: lethality, anaesthesia and physiological effects.** *Aquarium Sciences and Conservation. 2(1):21-34*
ISSN: 1357-5325
The lethality, anaesthetic and physiological effects of 'pulsed' cyanide (CN^-) exposures to a common tropical marine fish *Dascyllus aruanus* were assessed. Cyanide (25 and 50 mg/l) was applied as pulses (10, 60 and 120 s) to fish under non-stressed and stressed (by chasing and/or placing fish under hypoxic stress) conditions. Following treatment, the time until recovery and the percent survival were determined. The fish were allowed a 2.5 week recovery period from the treatments at which time four physiological end-points were measured: (1) the blood haemoglobin content, (2) the percent blood O^2 content, (3) the liver rhodonase activity and (4) the liver O^2 consumption rate. The greater the CN^- concentration and exposure time, the longer the recovery time.
Descriptors: oxygen consumption, liver, biological stress, cyanides, marine fish, tropical fish, fish physiology, anaesthetics, *Dascyllus aruanus*, physiology, cyanide,

Harrell RM (1992) **Stress mitigation by use of salt and anesthetic for wild striped bass captured for brood stock.** *Progressive Fish Culturist.* 54(4):228-233
NAL Call No. 157.5 P94
Gravid striped bass (*Morone saxatilis*) were collected from spawning grounds of the Choptank River, Maryland, to evaluate ways of mitigating stress effects associated with capture and transport. Stress alleviation was attempted through the use of salt (10 g/L), anesthetic (tricaine (MS-222) at 25 mg/L), or a combination of the two. Two capture techniques were used, electrofishing and gill netting, and all captured fish were immediately placed in tanks with the respective treatment then transported by truck to the hatchery. Fish stress was measured by plasma corticosteroid and chloride levels. The times required for stressed fish to recover, as measured by a return to baseline values of plasma corticosteroids, indicated that stress was mitigated most effectively by salt alone. The combination of salt and anesthetic was second in effectiveness, and the anesthetic alone was least effective. Although fish in each treatment initially exhibited signs of hypochloremia, only those fish transported in anesthetic along exhibited long-term signs of hypochloremia regardless of capture method.
Descriptors: biological stress, anaesthetics, *Morone saxatilis*, salts, fish handling, fish culture

Hattingh J (1977) **The effect of tricaine methanesulphonate (MS-222) on the microhaematocrit of fish blood.** *Journal of Fish Biology.* 10(5):453-455
NAL Call No. QL614 J68
The effects of the anaesthetic, MS-222, on the microhaematocrit value of freshwater fish

have been examined. Blood containing MS-222 showed a higher haematocrit value than blood without the anaesthetic and haemolysis occurred in the former after a variable time depending on the concn. The results are discussed in relation to previous findings.
Descriptors: serological studies, anaesthetics, Pisces
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Hettiarachchi M, Senadheera SPSD (1999) **Efficacy of quinaldine sulphate as an anaesthetic for the ornamental carp (*Cyprinus carpio*) in simulated packaging for long distance transport.** *Sri Lanka Journal of Aquatic Sciences.* 4:13-22
ISSN: 1391-2038
The high cost incurred in transporting fish in large volumes of water is a major problem in airlifting ornamental fish to foreign markets. The present study was carried out to investigate the efficacy of the anaesthetic, quinaldine sulphate buffered with sodium bicarbonate on ornamental carps (Koi carps, *Cyprinus carpio*) in simulated packaging for air transport. Quinaldine sulphate significantly reduced the rate of oxygen consumption and the accumulation of ammonia in water. The most efficient concentration of buffered quinaldine sulphate which was responsible for the greatest reduction in accumulation of ammonia and the rate of oxygen consumption was 50 ppm. Young koi carps of 7.5-9.0 cm in total length anaesthetised with 50 ppm quinaldine sulphate at the density of 40% of fish body weight to weight of water ratio did not show any mortality at room temperature of 28°C during the 40 hours of exposure time while unanaesthetized fish at the same density suffered 100% mortality. The recovery time during the post-packaging period was found to be less than 5 minutes. The present study indicates that young koi carps could be transported at higher packing densities, using the suitable dosage of quinaldine sulphate which will maximize the effective utilization of space and weight during transportation.
Descriptors: anaesthetics, live storage, transportation, ornamental fish, survival, *Cyprinus carpio*, common carp
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Hignette M (1984) **The use of cyanide to catch tropical marine fish for aquariums and its diagnosis.** Comptes Rendus des Journees Aquariologiques de l'Institut Oceanographique, 16 Dec 198 (Proceedings of Marine Aquariology of the Oceanographical Institute, 16 Dec 1983.). *Oceanis. Serie de documents oceanographiques. Paris.* 10(5):585-591
ISSN: 0182-0745
Marine tropical fish for the pet industry are nowadays very often caught with cyanide sodium, which is used for its "anaesthetic" effect. This method however can be responsible for fish dying as much as several weeks after transport, and must be avoided. In order to stop exporters having recourse to this practice, fish importers and aquariologists must know how to measure cyanide themselves in fish or obtain analyses from reliable laboratories.
Descriptors: cyanides, aquaria, tropical fish, catching methods, fish poisoning, stupefying methods, mortality causes
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Hirata M, Isoda S, Kanao M, Shimizu H, Inoue S (1970) **Studies on anesthetics for fish.** *Bulletin of the Japanese Society for Scientific Fisheries* 36(11):1127-1135
NAL Call No. 414.9 J274
92 anthranilate derivatives were synthesized and examined for their anaesthetic effect on Carassius and puffer. Compound No. 36, DP-1166, was the most potent anaesthetic for these

fish and also showed excellent effectiveness on other spp.

Descriptors: anesthetics, DP-1166, Carassius, puffer, anthranilate derivatives

Hoffmann R, Lommel R, Riedl M (1982) **Influence of different anaethetics and bleeding methods on hematological values in fish.** *Archiv fur Fischereiwissenschaft.* 33(1-2):91-103

Erythrocyte, leucocyte and thrombocyte values, hemoglobin, PCV and differential blood cell counts were investigated in a cyprinid (*Carassius carassius* L.) and in a salmonid (*Salmo gairdneri* Richardson) fish using two different methods of blood sampling and four methods of anaesthesia. Whereas red blood cell and thrombocyte values were significantly altered both by anaesthesia and method of bleeding, such an influence could not be proved in leucocyte values.
Descriptors: anesthetics, blood cells, hematology, methodology, *Carassius carassius, Salmo gairdneri*

Houston AH, Corlett JT (1976) **Specimen weight and M.S. 222.** *Journal of the Fisheries Research Board of Canada.* 33(6):1403-1407

The influence of specimen wt upon induction of and recovery from Stage I M. S. 222 (ethyl m-aminobenzoate methanesulphonate) was examined in gold-fish (*Carassius auratus*), brook (*Salvelinus fontinalis*), and rainbow trout (*Salmo gairdneri*) exposed to various anesthetic concn-temp combinations. Both induction and recovery times varied inversely with wt, the influence of wt being particularly pronounced among smaller specimens. These observations are consistent with the hypothesis that attainment of 'critical' internal anesthetic concn is influenced by wt-specific variation in the relationship between gill area and extracellular phase vol.
Descriptors: anaesthetics, *Carassius auratus, Salvelinus fontinalis, Oncorhynchus mykiss*

Hovda J, Linley TJ (2000) **The potential application of hypothermia for anesthesia in adult Pacific salmon.** *North American Journal of Aquaculture.* 62(1):67-72

We subjected 190 adult pink salmon *Oncorhynchus gorbuscha* to water temperatures of -1.5, -3.0°C, -4.5°C, and -6.0°C to evaluate the potential of hypothermia for anesthesia. The temperatures were obtained by dissolving salt (NaCl) at concentrations ranging from 25ppt to 90ppt and recirculating the solutions through a thermostatically controlled chiller. The time to each anesthetic stage (sluggishness, loss of movement, and complete anesthesia) declined with decreasing temperature but did not differ significantly between sexes. The most rapid change in response occurred between -1.5°C and -3.0°C. Time to recovery was also influenced by temperature and was directly related to the time to complete anesthesia. In contrast, anesthesia temperature had no affect on egg survival, nor was there a significant difference in survival between the experimental groups and the control (CO^2). We conclude that hypothermia is effective for short-term anesthesia of Pacific salmon *Oncorhynchus spp.* for spawning but note that its application for iteroparous or freshwater stenohaline species may be problematic because of the physiological effects induced by cold shock and exposure to high salinity. Further work will also be needed to determine its utility for large-scale operation.

Descriptors: temperature tolerance, hypothermia, fish culture, survival, anaesthesia, *Oncorhynchus gorbuscha*, pink salmon
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Howe GE, Bills TD, Marking LL (1990) **Removal of benzocaine from water by filtration with activated carbon.** *Progressive Fish Culturist.* 52(1):32-35
NAL Call No. 157.5 P94
Benzocaine is a promising candidate for registration with the U.S. Food and Drug Administration for use as an anesthetic in fish culture, management, and research. A method for the removal of benzocaine from hatchery effluents could speed registration of this drug by eliminating requirements for data on its residues, tolerances, detoxification, and environmental hazards. Carbon filtration effectively removes many organic compounds from water. This study tested the effectiveness of three types of activated carbon for removing benzocaine from water by column filtration under controlled laboratory conditions. An adsorptive capacity was calculated for each type of activated carbon. Filtrasorb 400 (12 x 40 mesh; U.S. standard sieve series) showed the greatest capacity for benzocaine adsorption (76.12 mg benzocaine/g carbon); Filtrasorb 300 (8 x 30 mesh) ranked next (31.93 mg/g); and Filtrasorb 816 (8 x 16 mesh) absorbed the least (1.0 mg/g). Increased adsorptive capacity was associated with smaller carbon particle size; however, smaller particle size also impeded column flow. Carbon filtration is a practical means for removing benzocaine from treated water.
Descriptors: water filtration, anaesthetics, aquaculture effluents, wastewater treatment, water quality control, hatcheries, biofilters, water quality, aquiculture, activated carbon, effluent treatment, water treatment, filtration, benzocaine, activated carbon
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Hseu JR, Yeh SL, Chu YT, Ting YY (1995) **Application of sodium bicarbonate and sulfuric acid for anesthetization of black porgy *Acanthopagrus schlegeli*.** *Journal of Taiwan Fisheries Research.* 3(2):151-159
NAL Call No. SH1 S48
Solutions of sodium bicarbonate ($NaHCO_3$) and sulphuric acid (H_2SO_4) were mixed in seawater to try to anesthetize black porgy (Acanthopagrus schlegeli). The results indicated that the anesthetic induction time was correlated positively with body size and negatively with the concentration of $NaHCO_3$ and H_2SO_4, but only the concentration influenced the recovery time. 675 ppm $NaHCO_3$, mixed with 395 ppm H_2SO_4, might be suitable to anesthetize black porgy because the induction time and the recovery time of most of the fish were less than 6 minutes at this concentration. No fish died during anesthetization and 5 days thereafter. Thus, this technique should be effective and safe for anesthetization of black porgy. An examination was also made of the changes of the physiological parameters of black porgy after anesthetization by 675 ppm $NaHCO_3$, mixed with 395 ppm H_2SO_4. The values of hematocrit, hemoglobin, plasma glucose, and total plasma protein were not significantly different between the anesthesia and the control groups. However, the plasma chloride ion concentration decreased significantly and the osmolarity increased significantly after anesthetization.
Descriptors: anaesthesia, sulphuric acid, fish physiology, *Acanthopagrus schlegeli*, methodology, sodium bicarbonate
ASFA; Copyright © 2003, FAO

Hseu Jinn-Rong, Yeh Shinn-Lih, Chu Yeong-Torng, Ting Yun-Yuan (1996) **Influence of the anesthetic, 2-phenoxyethanol, on hematological parameters of black porgy *Acanthopagrus schlegeli*.** *Journal of Taiwan Fisheries Research.* 4(2):127-132
NAL Call No. SH1 S48
In this study, an examination was made of changes of hematological parameters in black porgy *Acanthopagrus schlegeli* after anesthetization in 2-phenoxyethanol (2-PE) of various concentrations. In 400 and 600 ppm 2-PE solutions, black porgies were anesthetized to total loss of equilibrium within 3 min. All values of hematological parameters of the anesthetized fishes were not significantly different between the anesthesia and control groups. In 200 ppm 2-PE solution, since the fish could not be anesthetized to total loss of equilibrium within 30 min, the black porgies which were anesthetized for 15 min in this solution were tested and it was found that the fish showed a significant decrease in hematocrit and increase in plasma glucose. It is therefore concluded that higher dosages of 2-PE will take less time to induce less stress effect on the anesthetized fish.
Descriptors: fish culture, biological stress, aquaculture techniques, fish physiology, anaesthetics, haematology, anaesthesia, *Acanthopagrus schlegeli*
ASFA; Copyright © 2003, FAO

Hseu Jinn-Rong, Yeh Shinn-Lih, Chu Yeong-Torng, Ting Yun-Yuan (1994) **The use of 2-phenoxyethanol as an anesthetic in the transport of black porgy Acanthopagrus schlegeli.** *Journal of Taiwan Fisheries Research.* 3(1):11-18
NAL Call No. SH1 S48
A study was conducted to investigate the application of 2-phenoxyethanol (2-PE) in a closed transport system involving black porgy (*Acanthopagrus schlegeli*) in polyethylene bags. After 24 h sealed packaging, addition of 50-200 ppm 2-PE reduced the accumulation of total ammonia-nitrogen of sea water in the bags containing fish. However, 2-PE could not prevent acidification of the sea water in the bags. In another experiment, effects of 2-PE on the changes of hematological parameters in black porgy were examined at 0, 12, and 24 h after sealed packaging. The results indicated that packaging time and anesthetic did not affect the values of hematocrit, hemoglobin and osmolarity. The value of serum glucose was the only changed parameter during this experiment. The values of serum glucose increased following the duration of packaging time. The 2-PE added group had lower average values of serum glucose than the control group, but the differences between two groups were not significant. Considering the effect of 2-PE on the reduction of the accumulation of total ammonia-nitrogen in sea water and serum glucose of fish during sealed packaging as well as its cheap price, 2-PE was recommended for application in transport of fish.
Descriptors: live storage, transportation, anaesthetics, haematology, fish culture, *Acanthopagrus schlegeli*
ASFA; Copyright © 2003, FAO

Hseu Jinn-Rong, Yeh Shinn-Lih, Chu Yeong-Torng, Ting Yun-Yuang (1994) **The anesthetic effect of 2-phenoxyethanol in goldlined sea bream (*Sparus sarba*).** *Journal of Taiwan Fisheries Research.* 2(2):41-49
NAL Call No. SH1 S48
Responses of goldlined sea bream (*Sparus sarba*) to the anesthetic 2-phenoxyethanol (2-PE) were investigated. The induction time (INT) and recovery time (RET) increased when anesthesia reached the later stages. INT increased also with lower concentration of 2-PE, while RET showed the opposite. However, larger fish took longer INT, but RET did not show the other way. Different intervals between two anesthesias resulted different INT

(INT2) of the second anesthesia in comparison with that of the first INT (INTI). At 0 or 5 minutes intervals, INT2 was shorter than INT1. As opposite to the above findings, INT2 was longer than INTI at 30 minutes, 1 hour or 6 hours of interval. At 24 hours of interval, half of the anesthetized fish had longer INT2 and the rest of fish had shorter INT2. The results indicated that the anesthetic sensitivity of goldlined sea bream responding to 2-PE would revert after 24 hour's recovery from first anesthesia.
Descriptors: anaesthetics, anaesthesia, *Sparus sarba*, evaluation
ASFA; Copyright © 2003, FAO

Hseu Jinn-Rong, Yeh Shinn-Lih, Chu Yeong-Torng, Ting Yun-Yuen (1994) **The changes of hematological parameters during sustained anaesthesia with 2-phenoxyethanol in yellowfin porgy (*Acanthopagrus latus*).** *Journal of Taiwan Fisheries Research.* 2(2):63-68
NAL Call No. SH1 S48
Yellowfin porgy (*Acanthopagrus latus*) was anaesthetized with 2-phenoxyethanol, and sampled at 0, 1, 6, 12, 24 h during anesthesia and 24 h after recovery. Sampling time and anesthetic were found to affect the values of hematological parameters. Most of the time, the control group had higher average values of hematocrit and hemoglobin than the anesthesia group, but the difference between two groups was not significant except in hematocrit values at 24 h during anesthesia. Both sets of values of two groups reached the least level at 12 h, and gradually recovered to starting point. As to serum glucose, the anesthesia group had higher average values than the control group from 1 to 12 h during anesthesia, however, the difference between two groups was significant only at 6 h. The values of serum glucose in the anesthesia as well as the control group decreased from starting point, and never recovered even after 24 h recovering.
Descriptors: anaesthesia, anaesthetics, haematology, fish physiology, *Acanthopagrus latus*
ASFA; Copyright © 2003, FAO

Huish MT (1972) **Some responses of the brown bullhead to MS-222.** *Progressive Fish Culturist.* 34(1):27-32
NAL Call No. 157.5 P94
The effect of the anaesthetic MS-222 on the brown bullhead, *Ictalurus nebulosus*, at different temp and conc of anaesthetic, was investigated. Deep stages of anaesthesia were produced at 100 ppm in a relatively short time. The rate of induction of anaesthesia was not clearly related to size of fish. Mortalities were increased and recovery rates slowed at 22° and 27°C, as compared to 7°C, 12°C and 17°C, when treated for 1 hr with 75 and 100 ppm. Fish exposed for 1 hr to 100 ppm at 27°C for < 12 min survived. Those exposed for > 12 min died.
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Descriptors: MS-222, brown bullhead, *Ictalurus nebulosus*, anesthesia, dosage

Imamura-Kojima H, Takashima F, Yoshida T (1987) **Absorption, distribution and excretion of 2-phenoxyethanol in rainbow trout.** *Bulletin of the Japanese Society for Scientific Fisheries.* 53(8):1339-1342
NAL Call No. 414.9 J274
The absorption, distribution and excretion of 2-phenoxyethanol, which is a piscine anesthetic, were examined in rainbow trout (*Salmo gairdneri*). In fish tranquilized with a sate concentration of 2-phenoxyethanol, it was distributed in the brain, liver, kidney, and gall bladder, especially, the cerebellum. The 2-phenoxyethanol was rapidly excreted and the biological half-life under these experimental conditions was approximately 30 min.

Descriptors: bioaccumulation, anesthetics, excretion, *Salmo gairdneri*, phenoxyethanol
ASFA; Copyright © 2003, FAO

Itazawa Y, Takeda T (1982) **Respiration of carp under anesthesia induced by mixed bubbling of carbon dioxide and oxygen.** *Bulletin of the Japanese Society for Scientific Fisheries.* 48(4):489-493
NAL Call No. 414.9 J274
Anesthetization of fish by mixed bubbling into the ambient water of carbon dioxide and oxygen is being tried as a method of live transport. Respiratory parameters were measured with carp (*Cyprinus carpio*) before, during and after anesthesia induced by 1:1 mixed bubbling of carbon dioxide and oxygen. During the anesthesia, oxygen content and oxygen saturation of the arterial blood was maintained at levels higher than or equal to the pre-anesthetic ones owing to very high pO_2 accompanied with elevated Ht and Hb in the blood, in spite of enormously high pO_2 which ought to reduce the oxygen affinity of the blood. Gill ventilation was also maintained at the pre-anesthetic level due to increased frequency of respiration, notwithstanding its reduced stroke volume. Oxygen consumption was reduced to one-half its pre-anesthetic level, accompanied with a sharp drop of oxygen utilization at the gills.
Descriptors: anesthesia, transportation, respiration, live storage, *Cyprinus carpio*
ASFA; Copyright © 2003, FAO

Iwama GK, Mcgeer JC, Pawluk MP (1989) **The effects of five fish anaesthetics on acid - base balance, hematocrit, blood gases, cortisol, and adrenaline in rainbow trout.** *Canadian journal of zoology / Journal canadien de zoologie.* 67(8):2065-2073
NAL Call No. 470 C16D
Some physiological aspects of five fish anaesthetics in rainbow trout (*Oncorhynchus mykiss*) were investigated. The effects of benzocaine, 2-phenoxyethanol, MS-222 (Sandoz), metomidate, and carbon dioxide gas (CO_2) on acid - base regulation, hematocrit, blood gases, and cortisol and adrenaline concentrations were determined in resting rainbow trout fitted with chronic catheters in the dorsal aorta. A severe hypoxia developed with the cessation of breathing in deep anaesthesia. This was accompanied by a rise in blood pCO_2 and adrenaline concentration, and a fall in blood pH. Blood bicarbonate concentrations remained unchanged and cortisol concentrations declined with time. There was a transient increase in hematocrit coinciding with the increase in adrenaline concentrations.
Descriptors: anesthetics, fish physiology, hematology, steroids, hypoxia, *Oncorhynchus mykiss*
ASFA; Copyright © 2003, FAO

Iwama GK, Yesaki TY, Ahlborn D (1991) **The refinement of the administration of carbon dioxide gas as a fish anesthetic: The effects of varying the water hardness and ionic content in carbon dioxide anesthesia.** *ICES (International Council for the Exploration of the Sea) Council Meeting Papers., ICES, Copenhagen (Denmark).* 29 pp
The results indicate that two of three treatments that involved the addition of $NaHCO_3$ were capable of reducing the amount of stress experienced by juvenile steelhead (*Oncorhynchus mykiss*) when anesthetized by CO_2. Treatment 3, $NaHCO_3$ only, had significantly lower cortisol, lactate, and Hct values versus the control treatment. Consequently, it was concluded that the fish in treatment 3 exhibited the lowest overall stress responses to CO_2 anesthesia. The fish in treatment 9, $NaHCO_3$ and NaCl, had significantly lower cortisol and Hct values and was determined to be the next most effective treatment in reducing the stress associated

with CO_2 anesthesia. Treatments 3, 6, and 9 had water pH levels that were comparable to that of the water in which the fish were originally held. "Hyperactivity" was observed to be somewhat reduced when $NaHCO_3$ was added to the anesthetic bath water.
Descriptors: anaesthesia, carbon dioxide, biological stress, *Oncorhynchus mykiss*
ASFA; Copyright © 2003, FAO

Jeney Z, Jeney G, Olah J, Siwicki A, Danko I (1986) **Propanidid, a new anaesthetic for use in fish propagation.** *Aquaculture.* 54(1-2):149-156
NAL Call No. SH1A6
Propanidid, 3-methoxy-4-(N,N-diethyl-carbamoyl-methoxy)-phenylacetic acid n-propyl ester, was applied during artificial propagation of common carp, *Cyprinus carpio* L., and compared with MS 222. Primary and secondary stress effects from Propanidid and MS 222 were characterized by measuring the plasma adrenaline and noradrenaline levels, haemoglobin, blood glucose, plasma Ca^{++} and Cl^- concentrations, haematocrit and leucocrit values and activity of transaminases (GOT, GPT) in plasma. General effects of handling stress during propagation were reflected by significant hyperglycemia, decreasing Ca^{++} and Cl^- concentrations, and a significant increase in transaminase activity in plasma. Both anaesthetics decreased handling stress, though MS 222 caused a greater increase of catecholamines and of GPT and decrease of leucocrit and plasma Ca^{++}. Results of artificial propagation were similar in the two experimental groups.
Descriptors: anesthetics, fish culture, biological stress, fish handling, transportation, *Cyprinus carpio*
ASFA; Copyright © 2003, FAO

Jennings CA, Looney GL (1998) **Evaluation of two types of anesthesia for performing surgery on striped bass.** *North American Journal of Fisheries Management.* 18(1):187-190
NAL Call No. SH219.N66
Tricaine (MS-222) is the most widely used anesthetic for fishes, but induction and recovery times are rather long. Studies on salmonids have shown that electroanesthesia is a good alternative to MS-222 for short term (<1 min) immobilization. However, data on longer-duration (3-5-min) immobilization needed for surgical procedures are lacking. We analyzed induction and recovery times for 20 adult (52-81-cm) striped bass *Morone saxatilis* immobilized with electroanesthesia and MS-222. We defined induction time as the interval from the onset of each treatment until the fish was immobilized (i.e., did not respond to tactile stimuli) and recovery time as the interval from the fish's return to the water to its resumption of normal swimming. Surgical procedures similar to those necessary to implant a radio transmitter were performed on each fish. Induction time for fish immobilized with electroanesthesia (geometric mean, 8 s; 95% confidence interval [CI], 3-21 s) was much shorter than that for fish immobilized with MS-222 (geometric mean, 47 s; 95% CI, 38-58 s) ($P = 0.0006$). Additionally, fish immobilized with electroanesthesia recovered much faster (geometric mean, 9 s; 95% CI, 4-19 s) than fish immobilized with MS-222 (geometric mean, 206 s; 95% CI, 156-272 s) (P less than or equal to 0.0001). Faster induction and recovery times of fish immobilized with electroanesthesia and the ability to process more fish per unit time are major benefits of this technique.
Descriptors: anaesthesia, fishery biology, tagging, fishery management, *Morone saxatilis*, surgery, rockfish, mortality, striped bass
ASFA; Copyright © 2003, FAO

Josa Serrano A, Espinosa Velazouez E, Esteban Alonso J, del Nino Jesus A, Osanz Castan E (1993) **Use of anaesthetic 2-phenoxyethanol in carps (*Cyprinus carpio*): Levels blood concentration.** *Actas Del IV Congreso Nacional De Acuicultura., Centro De Investigaciones Marinas, Pontevedra (Spain).* pp. 731-736
In this work we studied the 2-Phenoxy ethanol concentration levels in the blood of Carp (*Cyprinus carpio*) after its use as anesthesic at different time, concentrations, temperatures and exposition. Anaesthesia levels as well as induction and recovery times are defined.
Descriptors: fish culture, anaesthesia, serological studies, blood, temperature, *Cyprinus carpio*, 2-phenoxiethanol
ASFA; Copyright © 2003, FAO

Kahl MD, Jensen KM, Korte JJ, Ankley GT (2001) **Effects of handling on endocrinology and reproductive performance of the fathead minnow.** *Journal of Fish Biology.* 59(3):515-523
NAL Call No. QL614 J68
Anaesthesia with MS-222 followed by intra-peritoneal (ip) injection (with a 10% ethanol in corn oil carrier) of fathead minnow either as one or three (weekly) treatments did not affect survival, behaviour or secondary sexual characteristics of the fish. Fecundity of the fish, as indicated by fertility and hatching success, was also unaffected. Gonadal condition (relative gonad mass, histopathology) was not altered in either sex. Male and female plasma sex steroids (beta -oestradiol, testosterone, 11-ketotestosterone) and male vitellogenin concentrations were not significantly affected by the treatments. Females subjected to either ip treatment regime had significantly higher plasma vitellogenin concentrations than control females. However, based on previous data, this difference did not appear to be treatment-related. Overall, exposure of fathead minnows to chemicals via the ip route should not confound the interpretation of toxicity tests with potential endocrine disrupting chemicals.
Descriptors: reproductive behavior, methodology, fish handling, anaesthetics, survival, bait culture, behaviour, secondary sexual characters, fecundity, fish physiology, animal behavior, sexual reproduction, toxicity, *Pimephales promelas*, fathead minnow, fathead minnows
ASFA; Copyright © 2003, FAO

Kaiser H, Vine N (1998) **The effect of 2-phenoxyethanol and transport packing density on the post-transport survival rate and metabolic activity in the goldfish, *Carassius auratus*.** *Aquarium Sciences and Conservation.* 2(1):1-7
ISSN: 1357-5325
To test the hypothesis that the anaesthetic 2-phenoxyethanol would reduce the metabolic rate and allow for higher transport packing densities, goldfish (3.93 plus or minus 1.99 g) were transported for 48 h at 25, 50 and 75 fish per 500 ml combined with anaesthetic concentrations of 0, 0.25 and 0.35 ml/l. The anaesthetic did not affect the survival rate or the oxygen and ammonia concentrations. Thus, its use could not be recommended for the transport of goldfish. It is suggested that optimum packing densities be based on a minimum post-transport oxygen value of 4 mg/l for goldfish.
Descriptors: fish physiology, respiration, oxygen consumption, dissolved oxygen, live storage, transportation, ornamental fish, aquarium culture, freshwater fish, anaesthetics, *Carassius auratus*
ASFA; Copyright © 2003, FAO

Kaminski R, Myszkowski L, Wolnicki J (2001) **Response to 2-phenoxyethanol in juvenile *Vimba vimba* (L.).** *Archiwum rybactwa polskiego/Archives of Polish fisheries. Olsztyn* 9(1):71-78
NAL Call No. SH293 P7A73

In Poland, the anaesthetic effect of 2-phenoxyethanol on juvenile *Vimba vimba* (L.) aged 38-179 days post-hatch (26-56 mm total length) was studied at 25°C. The concentration which anaesthetized 100% of the fish within 10 min without causing mortality after 15 min of exposure ranged from 0.35 to 0.48 g/dm super(3) in 38 day-old vimba and from 0.33 to 0.43 g/dm super(3) in older fish. The induction and recovery times were shorter in the initial phase of vimba juvenile life than in older fish. In fish of the same age, induction time or recovery time did not depend on their size or condition (Fulton's coefficient). At 25°C, 2-phenoxyethanol at 0.40 g/dm super(3) may be used to efficiently and safely anaesthetize vimba juveniles.
Descriptors: alcohols, fry, juveniles, *Vimba vimba*, anaesthesia, anaesthetics, phenols, freshwater fish, freshwater aquaculture
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Kaneko K (1982) **On the removal of larger freshwater fishes.** *Biennial report of the Keikyu Aburatsubo Marine Park Aquarium. Miura.* 1981(11):39-45
Because of reconstruction of the tank where Arapaima and other freshwater fishes had been kept, it was necessary to temporarily remove them to another tank. Arapaima and Lepisosteus have the gas bladder well developed for aerial respiration. Some anesthetic tests were examined with such physostomous fish as Protopterus and Channa prior to the removal. In practical uses, 10% - Quinaldine was added to the water in concentration of 100 ppm. The 2 anesthesized Osteoglossum was removed at first by handling with a blanket, and the 3 species of catfish were succeeded in the same manner. The concentration amounted to 150 ppm. The 3 Mylopharyngodon was removed. Arapaima and Lepisosteus still kept their air-breathing, so 200 ppm-MS-222 and 20%-Fluothane were sprayed at their gills as a supplementaly anesthetic treatment.
Descriptors: aquaria, fish handling, anesthesia, freshwater fish
ASFA; Copyright © 2003, FAO

Kazun K, Siwicki AK (2001) **Propiscin -- a safe new anaesthetic for fish.** *Archiwum rybactwa polskiego/Archives of Polish fisheries. Olsztyn.* 9(2):183-190
NAL Call No. SH293 P7A73
Anaesthetics are needed when handling fish, especially during tagging. However, most anaesthetics applied at present have a strong toxic effect on fish. For this reason it is only permissible to keep fish anaesthetized for a short time. A new anaesthetic, Propiscin, which allows fish to be anaesthetized for up to 0.5 h, has been successfully tested in Poland. It contains a 0.2% stabilized solution of etomidate and can be used as a bath. When administered correctly, the required disappearance of sense perception and motor reflexes in the fish can be obtained in about 2-4 min. The low toxicity of this pharmacological confection has been proved according to a full set of clinical, toxicological, hematological and biochemical criteria. Clinical tests have been conducted on with many fish species, mainly salmonids.

Descriptors: anaesthetics, anaesthesia, freshwater aquaculture, pharmacology, drugs, fish culture, fish handling, toxicology, Propiscin
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Keene JL, Noakes DLG, Moccia RD, Soto CG (1998) **The efficacy of clove oil as an anaesthetic for rainbow trout, *Oncorhynchus mykiss* (Walbaum).** *Aquaculture Research.* 29(2):89-101
NAL Call No. SH1 F8

The anaesthetic effects of clove-oil-derived eugenol were studied in juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum). Acute lethality and the effects of multiple exposures to eugenol were measured. The estimated 8-96 h LC sub(50) for eugenol was found to be approximately 9 p.p.m. Times to induction and recovery from anaesthesia were measured and compared with MS-222 under similar conditions. Eugenol generally induced anaesthesia faster and at lower concentrations than MS-222. The recovery times for fish exposed to eugenol were six to 10 times longer than in those exposed to similar concentrations of MS-222. Clove oil eugenol was determined to be an acceptable anaesthetic with potential for use in aquaculture and aquatic research. Doses of 40-60 p.p.m. eugenol were found to induce rapid anaesthesia with a relatively short time for recovery in juvenile trout.
Descriptors: fish culture, anaesthetics, lethal effects, recovery, *Oncorhynchus mykiss*, eugenol, fish culture, anaesthetics

Klaverkamp JF, Lockhart WL, Metner WL, Grift N (1976) **Effects of chronic DDT/DDE exposure on anesthetic induction and recovery times in rainbow trout (*Salmo gairdneri*).** *Journal of the Fisheries Research Board of Canada.* 33(6):1331-1334
NAL Call No: 442.9 C16J
In rainbow trout (*S. gairdneri*) fed pellets containing 4.55{mu}g/g of p, p'-DDT and 6.81{mu}g/g of p, p'-DDE every other day. Anesthetic induction and recovery times of phenoxyethanol (PE) were less than those in trout fed control pellets. No interactions were observed in fish fed DDT/DDE and anesthetized with ethyl m-aminobenzoate methane sulphonate (M.S. 222). Differences observed between fish fed DDT/DDE and anesthetized with PE as compared to M.S. 222 could be due either to enhanced metabolism of PE or to the fact that PE and M.S. 222 have different modes or sites of action.
Descriptors: insecticides, DDT, anaesthetics, *Oncorhynchus mykiss*

Knoph MB (1995) **Effects of metomidate anaesthesia or transfer to pure sea water on plasma parameters in ammonia-exposed Atlantic salmon (*Salmo salar* L.) in sea water.** *Fish Physiology and Biochemistry.* 14(2):103-109
ISSN: 0920-1742
Atlantic salmon (*Salmo salar* L) postsmolts weighing 150 plus or minus 53 g were exposed to 14-15 mg/l TA-N (total ammonia-N) in sea water in 1 m super(3) tanks for 24h. Blood samples were then taken A) immediately after the fish were netted from the exposure tanks and stunned by a blow to the head; B) 2-20 min after the fish were transferred to 15 l of an anaesthetic solution of metomidate in “ammonia-free” sea water; or C) 2-20 min after the fish were transferred to 15 l of "ammonia-free" sea water. Plasma TA-N level was 18% lower in the anaesthetised fish compared to in the fish sampled directly from the exposure tanks (p less than or equal to 0.05), and accordingly 16% lower in the fish transferred to pure sea water although this difference was not significant (p = 0.07). Plasma glucose level was higher in the fish transferred to pure sea water than in the fish receiving the two other treatments (p less than or equal to 0.05), but plasma urea, osmolality, Na^+, Cl^-, Ca^{++} or Mg^{++} levels did not vary significantly between the difference treatments. Plasma TA-N level increased with time in the fish in the metomidate solution (p less than or equal to 0.02).
Descriptors: fish physiology, anesthesia, *Salmo salar*, blood, ammonia, hematology, biological sampling, metomidate

Kohbara J, Nanba K, Murachi S (1987) **The heart rate of carp anesthetized with tetraethylene glycol dibutyl ether.** *Bulletin of the Japanese Society of Scientific Fisheries.* 53(4):681
NAL Call No. 414.9 J274
An anesthetic effect on a carp (*Cyprinus carpio*) produced by tetraethylene glycol dibutyl ether was discovered. To clarify the physiological condition of carp under the anesthetic effect produced by this agent, the heart rate was measured during the anesthetic period and compared with the anesthetic effect produced by MS-222. Both MS-222 and tetraethylene glycol dibutyl ether produced an increase in the heart rate at first. The heart rate of the carp anesthetized with MS-222 maintained a high beat rate during the experiment. That affected by tetraethylene glycol dibutyl ether, however, fell off to below the heart rate of the fish in normal condition. It is estimated that the anesthetic mechanism of this agent is very different from that of MS-222.
Descriptors: heart, anesthesia, *Cyprinus carpio*, heart rate, tetraethylene glycol dibutyl ether
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Korstrom JS, Birtwell IK, Piercey GE, Spohn S, Langton CM, Kruzynski GM (1996) **Effect of hypoxia, fresh water, anaesthesia and sampling technique on the hematocrit values of adult sockeye salmon (*Oncorhynchus nerka*).** *Canadian technical report of fisheries and aquatic sciences/Rapport technique canadien des sciences halieutiques et aquatiques. Imprint varies.* 34 pp
ISSN: 0706-6457
The hematocrit values of adult sockeye salmon, *Oncorhynchus nerka*, were determined after the fish were subjected to sublethal hypoxia in salt water under simulated estuarine conditions, a residual oxygen bioassay, transfer from sea water to fresh water, and a lethal dose of the anaesthestic MS-222. Irrespective of treatment, hematocrit values determined in sequentially-collected aliquots of blood decreased in relation to the elapsed sampling time. A systematic and significant error occurred due to the ordinal number of sampling the hematocrit capillary tubes a maximum of three tubes of blood should be collected per fish for hematocrit determination. Hematocrit values of fish anaesthetized with 200 mg/L MS-222 were not significantly higher than those from control fish. Adult male and female sockeye salmon had similar hematocrit values. Hematocrit values were significantly increased after exposure to longer (48h) but not to shorter (6hr) term hypoxia. Hematocrit values were significantly elevated after 8.5 weeks, but not after 2.5 weeks residence in fresh water following transfer from sea water. The highest hematocrit values were from fish exposed to the residual oxygen bioassay which subjected the salmon to the greatest hypoxic stress. The utility of hematocrit values in relation to environmental stressors is discussed.
Descriptors: hypoxia, fresh water, anaesthesia, adults, haemoglobins, blood cells, oxygenation, bioassays, fish physiology, *Oncorhynchus nerka*
ASFA; Copyright © 2003, FAO

Kreiberg H (1992) **Metomidate sedation minimizes handling stress in chinook salmon.** Proceedings, 1992 Meeting of the Aquaculture Association Of Canada, 1-3 June, 1992, University of British Columbia, Vancouver, BC. *Bulletin of the Aquaculture Association of Canada. St. Andrews NB.* 92(3):52-54
NAL Call No. SH37.B8
Use of a recently introduced fish anaesthetic, metomidate, for controlling physiological response to stress from handling procedures in chinook salmon Oncorhynchus tshawytscha is described. Fish given a pre-handling exposure to metomidate responded to a crowding and netting procedure with relatively little elevation of plasma cortisol compared to other fish

handled without metomidate. Plasma cortisol in the metomidate-treated fish rose from resting level of 28.4 plus or minus 1.8 ng/mL to 61.0 plus or minus 7.5 ng/mL, compared to 204.9 plus or minus 16.2 ng/mL in untreated fish (mean plus or minus 2SE).
Descriptors: fish culture, fish handling, biological stress, therapy, drugs, *Oncorhynchus tshawytscha*, metomidate

Kreiberg H, Powell J (1991) **Metomidate sedation reduces handling stress in chinook salmon.** *World Aquaculture.* 22(4):58-59 ISSN: 1041-5602
NAL Call No. SH1.W62
Exposure to the recently introduced fish anaesthetic, metomidate, in the fishes' home culture container prior to a handling stress resulted in considerably reduced plasma corticosteroid response over untreated stressed fish. A practical and inexpensive procedure in use since 1989 is described for tanks, raceways and netpens. The unique sedative properties of metomidate are considered to have broad potential benefit to posthandling health and survival in sensitive fish such as chinook salmon. A requirement to handle live fish in order to carry out procedures such as transportation, grading, or benign or minor invasive sampling is recognized by many fish culturists as a potentially stressful experience which may result in reduced vigor or mortality in the affected stock. A number of researchers have described aspects of the physiological consequences of handling procedures used with temperate and coolwater fishes such as Pacific salmon species, various trout, striped bass and red drum. Essentially, the fright-flight response is triggered, involving adrenaline and subsequent impacts on the fish's ability to maintain its blood constituents, osmoregulation and immune system preparedness. Most reports have singled out the netting and capture phase of various procedures as the major contributor to overall stress associated with a particular procedure. Reports of the suitability of a recently introduced fish anaesthetic, metomidate, for minimizing the stress response to handling in striped bass and Atlantic cod suggested applications in the culture of Pacific salmon, particularly chinook salmon. Over a number of practical trials with different sizes of chinook and coho salmon, we developed a standard procedure for light sedation of fish before a handling disturbance. This report provides a summary and evaluation of our pre-handling technique. (DBO)
Descriptors: marine aquaculture, aquaculture techniques, fish physiology, *Oncorhynchus tshawytscha*

Kumlu M, Yanar M (1999) **Effects of the anesthetic quinaldine sulphate and muscle relaxant diazepam on sea bream juveniles (*Sparus aurata*).** *Israeli Journal of Aquaculture/Bamidgeh.* 51(4):143-147
NAL Call No. SH117 I75B36
In this investigation, the effects of an anesthetic, quinaldine sulphate (QS), and a muscle relaxant, diazepam (D), on sea bream juveniles (*Sparus aurata*) were studied. The application of diazepam significantly increased the safety level of the anesthetic QS for the sea bream juveniles. The fish entered light anesthesia at 5 ppm QS + 1 ppm D, as compared to 10 ppm QS. Similarly, the deep anesthesia level was reached at only 7.5 ppm QS + 1 ppm D as compared to 15 ppm QS. The use of QS alone at high concentrations (15-20 ppm) resulted in mortality of 30% to 100%. No mortality occurred in the fish treated with QS plus D at all anesthesia levels. Depending on the anesthetic concentrations used, the time to loss of balance and the recovery time were 0-2 min and 2-6 min, respectively. Administration of diazepam with a lower concentration of QS significantly enhanced the anesthesia, eliminated

the undesirable effects of QS and reduced the excitement and hyperactivity of the fish in the confined space, without leading to mortality. Suitable light and deep sedation stages of anesthesia for transportation and handling of sea bream juveniles (6-7 g) were obtained with dosages of 5 ppm QS + 1 ppm D and 7.5 ppm QS + 1 ppm D, respectively.
Descriptors: anaesthetics, fish culture, aquaculture techniques, fish larvae, survival, diazepam, anesthetics, quinaldine sulfate, *Sparus aurata,* quinaldine sulphate, diazepam, gilthead seabream
ASFA; Copyright © 2003, FAO

Ladu BMB, Ross LG (1997) **The effect of methods of immobilization on the haematology and tissue chemistry of rainbow trout *Oncorhynchus mykiss* Walbaum.** *Journal of Aquatic Sciences.* 12:31-41
ISSN: 0189-8779
The influence of methods of immobilization on the haematology and tissue chemistry of rainbow trout *Oncorhynchus mykiss* was investigated. Both chemical and electroanaesthesia had similar effects on haematology and tissue chemistry of the fish. Generally, however, haemoconcentration was minimized by electroanaesthesia and it is recommended as the preferred method of sampling because it is cheap, safe and less stressful. The demands on respiration, nutrient metabolism, ionoregulation and osmoregulatory activities were variously effected by each stressor.
Descriptors: haematology, fish physiology, tissues, histochemistry, anaesthesia, biological stress, *Oncorhynchus mykiss*
ASFA; Copyright © 2003, FAO

Laidley CW, Leatherland JF (1988) **Cohort sampling, anaesthesia and stocking-density effects on plasma cortisol, thyroid hormone, metabolite and ion levels in rainbow trout, *Salmo gairdneri* Richardson.** *Journal of Fish Biology.* 33(1):73-88
NAL Call No. QL614 J68
The effect of serial removal of fish from aquaria, anaesthesia and stocking density on plasma cortisol, thyroid hormone, metabolite and ion levels was examined in rainbow trout, Salmo gairdneri, to determine the consequences of normal handling and maintenance procedures on the activity of the pituitary-adrenal and pituitary-thyroid axes in the species.
Descriptors: *Salmo gairdneri, Oncorhynchus mykiss*, anaesthesia, fish handling, biological stress, fish physiology, stocking density
ASFA; Copyright © 2003, FAO

Laitinen M, Nieminen M, Pasanen P, Hietanen E (1981) **Tricaine (MS-222) induced modification on the metabolism of foreign compounds in the liver and duodenal mucosa of the splake (*Salvelinus fontinalis* X *Salvelinus namaycush*).** *Acta Pharmacologica et Toxicoogica.* 49(2):92-97
The splake, a popular game fish, is a crossbreed which must be reared in nurseries. The fish are marked under anaesthesia for later study. We anlayzed the effect of a common anaesthetic, tricaine (MS-222), on the metabolism of foreign compounds in the liver and duodenum of the splake. In the liver and to some extent in the duodenum, aryl hydrocarbon hydroxylase and epoxide hydrase activities were reduced during treatments. The ethoxycoumarin O-deethylase activities were not affected in either the liver or duodenum. Tricaine significantly decreased the hepatic UDP-glucuronosyltransferase activity. The decrease was greater when the aglycone used was p-nitrophenol than with methylumbelliferone. A similar effect was also found after trypsin treatment of the

microsomes. No significant decrease in the UDP-glucuronosyltransferase activity was detected in the duodenal mucosa. This was the case when both p-nitrophenol and methylumbelliferone were used as aglycones.
Descriptors: anaesthetics, enzymatic activity, liver, intestines, *Salvelinus fontinalis, Salvelinus namaycush*, MS-222

Lambooij E, Vis JW, van de Kloosterboer RJ, Pieterse C (2002) **Welfare aspects of live chilling and freezing of farmed eel (*Anguilla anguilla*): neurological and behavioural assessment.** *Aquaculture.* 210 (1/4):159-169
NAL Call No. SH1 A6
The overall objective of the study was to evaluate a slaughter method of eels, which consisted of chilling until their body temperature was <5°C for stunning, and subsequently placing them in cold brine at -18°C for 15 min for killing. Three distinct experiments and a control were performed. First, 19 eels with an average live weight of 758 ± 44 g were restrained and equipped with EEG, ECG electrodes and a temperature sensor inside the body. Then, they were placed in the ice water. Indices for the induction of unconsciousness and insensibility were the appearance of theta and delta waves and no response on pain stimuli, which disappeared at a body temperature of 8.0 ± 2.1°C after 12 ± 5 min in 15 eels. The responses to pain stimuli did not disappear in three eels. Within a confidence level of 95%, the percentage of eels that was not effectively stunned during the procedure in ice water of <5 °C was at least 5%. The heart rate decreased from 24 ± 10 beats/min (n=14) to 7 ± 4 (n=11) and became irregular during cooling down. When placed in the brine water of -18°C, the EEG showed rapid and extreme depolarization of the membranes, which started after 27 ±17 seconds (n=18). The ECG showed fluttering of the heart in all eels. None of the eels recovered after this procedure. For 10 eels with an average live weight of 128 ± 27 g, it was observed that the body temperature decreased from 17.1 ± 0.6 to 4.0 ± 0.5°C in the ice water. After 15 min in the brine water of -16.1 ± 2.2°C, the body temperature decreased to -3.1 ± 2.3°C. Finally, three groups of 7 eels and 8 single eels were placed in ice water of -0.0 ± 0.1°C. The observation of unrestrained eels revealed four phases. Animals were (1) swimming around in the water, (2) attempting to escape from the ice water, (3) pressing their nose to the wall or corner while showing clonic muscle cramps, and finally (4) breathing only, while all other muscle activity was totally suppressed. Afterwards, they were transferred to cold brine at -18°C, and none of the eels recovered. The eight control eels, which were transferred to water at 18°C swam around, except for one that was lying in an S-shape position at the bottom. After 570 and 605 seconds, two eels tried to escape from the box. The obtained results showed that the eels, which were transferred from water at 18°C to ice water, might be stressed, a specific behaviour and an irregular heart rate were observed. From an animal welfare point of view, it is therefore not recommended to stun eels by live chilling. Moreover, at least 5% of the eels will not be stunned at a body temperature of <5°C. Placing eels in brine water of -18°C is an effective method to kill the eels before slaughter. However, it cannot be recommended to place conscious eels in cold brine water, because it takes more than 27 seconds before unconsciousness may be induced.
Descriptors: animal behaviour, animal welfare, body temperature, chilling, freezing, heart rate, neurology, pain, slaughter, stunning, eels, Anguilla, Anguillidae, Anguilliformes, Osteichthyes, fishes, diadromous fishes, aquatic animals, aquatic organisms

Le Bras YM (1982) **Effects of anaesthesia and surgery on levels of adrenaline and noradrenaline in blood plasma of the eel (*Anguilla anguilla* L.).** *Comparative Biochemistry and Physiology, C.* 72C(1):141-144
NAL Call No. QP1 C6
The effects of surgery and anaesthesia on adrenaline and noradrenaline plasma levels were investigated in the eel (*Anguilla anguilla* L.). Effect of surgery: highest values were obtained when putting back the fish in water. Three hours after surgery, adrenaline and noradrenaline plasma levels were always significantly higher than those obtained 24 and 48 hr after surgery. Effect of anaesthesia: anaesthesia only had no effect on adrenaline and noradrenaline plasma levels. It was concluded that the trauma of surgery was mainly responsible for the elevation of CA plasma levels in the eel. A minimum post-operative period of 24 hr should be allowed before any blood sampling for estimation of resting CA plasma levels. Resting adrenaline and noradrenaline plasma levels, 48 hr after surgery, were respectively 1.31 ± 0.38 and 3.37 ± 0.41 pmol/ml.
Descriptors: anaesthesia, biological stress, blood, *Anguilla anguilla*, adrenaline, noradrenaline
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Lewis DH, Tarpley RJ, Marks JE, Sis RF (1985) **Drug induced structural changes in olfactory organ of channel catfish *Ictalurus punctatus*, Rafinesque.** *Journal of Fish Biology.* 26(3):355-358
NAL Call No. QL614 J68
The fish anesthetic tricaine methanosulfate destroyed the cilia on olfactory sensory epithelia of channel catfish (*I. punctatus*) when fish were exposed to tranquilizing doses of the drug. Cilia on the nonsensory epithelium appeared to be unaffected by multiple exposures of the drug. Sensory cilia regenerated within 28 days after exposure.
Descriptors: anesthetics, histopathology, olfactory organs, *Ictalurus punctatus*
ASFA; Copyright © 2003, FAO

Limsuwan C, Grizzle JM, Plumb JA (1983) **Etomidate as an anesthetic for fish: Its toxicity and efficacy.** *Transactions of the American Fisheries Society.* 112(4):544-550
NAL Call No. 414.9 AM3
Etomidate was tested as an anesthetic on channel catfish Ictalurus punctatus, golden shiners Notemigonus crysoleucas, and bluegills Lepomis macrochirus. The 24-hour median lethal concentration (LC50) at various temperatures was lowest for bluegills (0.61-0.68 mg/liter) and highest for golden shiners (1.87-2.73 mg/liter). Generally, etomidate was more toxic, and induction of anesthesia and recovery from anesthesia were slower, at 17°C than at 22 °C and 27 °C. Both channel catfish and golden shiners were anesthetized within 15 minutes by 3 mg/liter, and all of the fish survived at this concentration for 30 minutes. concentrations of 0.2 and 0.4 mg/liter sedated channel catfish, and 0.4 and 0.6 mg/liter sedated golden shiners. The safety index LC50/EC50 (median effective concentration) for anesthesia of channel catfish and golden shiners at 22°C ± 1°C decreased from 6.2 to 5.0 and 7.6 to 4.0, respectively, as exposure length increased from 10 to 80 minutes.
Descriptors: anesthetics, toxicity, *Ictalurus punctatus, Notemigonus crysoleucas*, *Lepomis macrochirus*, utilization, evaluation, etomidate
ASFA; Copyright © 2003, FAO

Limsuwan C, Limsuwan T, Grizzle JM, Plumb JA (1983) **Stress response and blood characteristics of channel catfish (*Ictalurus punctatus*) after anesthesia with etomidate.**

Canadian Journal of Fisheries and Aquatic Sciences. 40(12):2105-2112
NAL Call No: 442.9 C16J
Continuous anesthesia of channel catfish (*Ictalurus punctatus*) with 0.6 mg/L etomidate for 96 h caused a small but statistically significant decrease in plasma protein concentration at all sampling periods. Anesthetized fish were not stressed by the periodic sampling. Fish anesthetized with 3 mg/L etomidate and then confined in a net for 10 min had reduced plasma cortisol response and no significant plasma glucose increase compared with unanesthetized controls. Anesthesia did not prevent hyperchloremia that developed 3 h after the 10-min confinement. No histological changes were found in fish anesthetized with etomidate. Anesthesia with etomidate before netting could be useful when handling fish because of the reduced stress response.
Descriptors: anaesthesia, fish culture, haematology, histology, biological stress, *Ictalurus punctatus*
ASFA; Copyright © 2003, FAO

MacAvoy SE, Zaepfel RC. (1997) **Effects of tricaine methanesulfonate (MS-222) on hematocrit: First field measurements on blacknose dace.** *Transactions of the American Fisheries Society.* 126(3):500-503
NAL Call No. 414.9 AM3
Tricaine methanesulfonate (MS-222) is an anesthetic commonly used to reduce fish stress during transport or sampling. The exposure of blacknose dace Rhinichthys atratulus to MS-222 at concentrations of 300 mg/L or 500 mg/L for 9 min or less did not raise hematocrit levels above those of controls. Hematocrit levels tended to be somewhat lower in experimental groups than in controls; however the only significant difference ($P = 0.044$) among treatments occurred between the control fish (35% ± 2.3; N = 6) and fish exposed to 500 mg/L for 3 min (30% ±1.8; N = 7). This difference suggests that initial exposure to MS-222 may cause stress or, less likely, that the anesthetic has some inherent hemodilution effect. Workers who monitor environmental acidification may be concerned with the hematocrit of the acid-sensitive blacknose dace because hematocrit increases during acid stress. The use of MS-222 to ease hematocrit sampling should not elevate measurements.
Descriptors: anaesthetics, blood, biological stress, therapy, acidification, pollution indicators, anesthetics, hematocrit, stress, USA, Virginia, Blue Ridge, Paine Run, tricaine methanesulfonate, *Rhinichthys atratulus*, USA, Virginia, Blue Ridge Mts., Paine Run, therapy, Blacknose dace
ASFA; Copyright © 2003, FAO

MacKinlay DD, Johnson MVD, Celli DC (1994) **Evaluation of stress of carbon dioxide anaesthesia.** *High Performance Fish: Proceedings of an International Fish Physiology Symposium at the University of British Columbia in Vancouver, Canada, July 16-21 1994.* Fish Physiology Association, Vancouver, BC (Canada). pp. 421-424
NAL Call No. QL639.1 I58 1994
The Salmonid Enhancement Program (SEP) has applied coded-wire tags to 7-12 million fish per year since the early 1980's, an operation that requires short-term (5-10 min) anaesthesia. Tricaine methane sulphonate (TMS) and 2-phenoxyethanol (TPE) were the chemicals most often used in the early years of the SEP. Several concerns, including the safety of hatchery workers using TPE, warnings about releasing fish into seawater soon after exposure to TMS and increasing restrictions on the availability of drugs for routine procedures has led to a switch to carbon dioxide gas as the preferred anaesthetic at SEP hatcheries. One of the marked differences between anaesthesia with carbon dioxide and other anaesthetics is that

fish exhibit an extreme hyperactive response when first immersed in water containing a high concentration of carbon dioxide. This led to concerns that the fish were extraordinarily stressed by carbon dioxide anaesthesia, which led to this series of experiments to determine if the carbon dioxide caused a greater degree of measurable physiological stress than the other anaesthetics. Cortisol was chosen as the index of stress, in accordance with common practice (Donaldson, 1981).
Descriptors: biological stress, tagging, anaesthesia, carbon dioxide, fish physiology, fish handling, Salmonidae, fish culture
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Malmstroem T, Salte R, Gjoeen HM, Linseth A (1993) **A practical evaluation of metomidate and MS-222 as anaesthetics for Atlantic halibut (*Hippoglossus hippoglossus* L.).** *Aquaculture.* 113 (4):331-338
NAL Call No. SH1A6
Metomidate and MS-222 were tested as anaesthetics for Atlantic halibut (*Hippoglossus hippoglossus* L.) at temperatures of 9.5 ±0.3°C and 10.5 ± 0.3°C. An effective concentration of the drug was defined as one giving a sure state of anaesthesia for 75% of the fish after an exposure time of less than 5 min. The lowest effective concentration of metomidate was 10 mg/l; with MS-222, concentrations of 250 mg/l were required. Doses should further be kept below 60 mg metomidate/l or 480 mg MS-222/l. There was interaction between anaesthetic and dose for both drugs with respect to the required exposure time, which focuses on the necessity of testing several doses when a new anaesthetic is to be used on a given species. In is concluded that metomidate gives a broader safe anaesthetic range with a lower effective dose than MS-222.
Descriptors: fish culture, aquaculture techniques, pharmacology, anaesthetics, comparative studies, *Hippoglossus hippoglossus*, metomidate, MS-222
ASFA; Copyright © 2003, FAO

Marx H, Brunner B, Weinzierl W, Hoffmann R, Stolle A (1996) **Comparative investigations on different methods for stunning fish with special regard to meat quality parameters.** *Proceedings of the conference of IIR Commission C2, Bordeaux Colloquium -- Refrigeration and Aquaculture. Froid et Aquaculture -- Colloque de Bordeaux, compte-rendu de la reunion de la Commission C2 de l' IIF.* pp. 199-206
Taking into account aspects of meat quality and animal welfare, three methods for stunning fish were compared: manually (blow on the head, stab in the neck) with electricity and using CO_2. The following results were obtained for eel (n = 72), carp (n = 120) and trout (n = 54). From the view of animal welfare, the effects on the fish were judged. Excitation and mucus secretion, as well as the period of lime until the fish were in anaesthesia were recorded. With manual and electrical stunning, all fish were anaesthetized almost immediately, while using CO_2, it takes 3.2 min (trout), 9.2 min (carp) and 109.7 min (eel), on average. After slaughter, after three and eight days of storing the fish on ice, the meat quality parameters, pH value, water holding capacity and rigor mortis were measured. CO_2 stunning showed the lowest pH-values and water holding capacities; also, rigor mortis in carp and eel advanced most. Testing of raw and prepared fish was performed by a sensoric team. In many cases, fish anaesthetized manually were ranked better than the other groups. The findings indicate that CO_2 was not appropriate for stunning carp and eel. Electrical stunning, with some improvements, could meet the requirements of meat quality and animal welfare.

Descriptors: processing fishery products, anaesthesia, slaughter, quality, *Oncorhynchus mykiss, Cyprinus carpio, Anguilla anguilla*
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Massee KC, Rust MB, Hardy RW, Stickney RR (1995) **The effectiveness of tricaine, quinaldine sulfate and metomidate as anesthetics for larval fish.** *Aquaculture.* 134(3-4):351-359
NAL Call No. SH1A6
Tricaine, quinaldine sulfate, and metomidate were compared as anesthetics for larvae of two species of fish, red drum (*Sciaenops ocellatus*) and goldfish (*Carassius auratus*). Larvae were exposed to various concentrations of each anesthetic and the percentages of fish reaching stage 4 of anesthesia, post-exposure recovery, and survival were recorded. Effective concentrations were defined as those which induced stage 4 of anesthesia within 3 min after exposure with a recovery time of 10 min or less. Post-exposure survival of 100% was an additional criteria used to define effective anesthetic concentrations. The lowest effective concentration of tricaine for red drum was 55 mg/l (26°C), while 50 mg/l (24°C) was the lowest effective concentration for goldfish. The lowest effective concentration of quinaldine sulfate for red drum was 35 mg/l (26°C), while 60 mg/l (24°C) was the lowest effective concentration for goldfish. Metomidate was found to be an ineffective anesthetic for both red drum and goldfish larvae based upon survival and recovery times. Mortality occurred in red drum larvae at all tested concentrations of metomidate. Larvae of both species that survived anesthesia with metomidate had longer induction and recovery times compared to larvae exposed to tricaine and quinaldine sulfate.
Descriptors: fish larvae, anesthetics, survival, *Sciaenops ocellatus, Carassius auratus*, tricaine, quinaldine sulfate, metomidate
ASFA; Copyright © 2003, FAO

Matthews GM, Paasch NN, Achord S, McIntyre KW, Harmon JR (1997) **A technique to minimize the adverse effects associated with handling and marking salmonid smolts.** *Progressive Fish-Culturist*. 59(4):307-309
NAL Call No. 157.5 P94
A system that allows anesthetization of juvenile salmonids before netting during a handling and marking operation is described. Our purpose for designing the system was to reduce or minimize any debilitating effects associated with these activities. When compared with smolts handled and marked in the traditional manner, use of the technique resulted in a significant reduction in the mortality of naturally migrating smolts of spring-summer chinook salmon Oncorhynchus tshawytscha during a posthandling and marking seawater challenge performance test. The treatment resulted in lower, but not significantly lower, plasma cortisol levels; however, sample sizes may have been too small for statistical verification. With a little ingenuity, the technique should be adaptable to most smolt handling or marking operations.
Descriptors: smolts, aquaculture techniques, marking, anaesthesia, fish culture, fish handling, biological stress, *Oncorhynchus tshawytscha*
ASFA; Copyright © 2003, FAO

Mazur CF, Boreham A, McLean W, Iwama GK (1991) **Rectified wide band white noise as an electroanaesthesia waveform for use with rainbow trout (*Oncorhynchus mykiss*).** *ICES Council Meeting Papers., ICES, Copenhagen (Denmark).* 28 pp.
Rectified and pulsed wide band white noise shows promise as an effective waveform for use in salmonid electroanaesthesia. The use of electroshocking for the capture of fish has often

resulted in muscular tetany and vertebral displacement leading to paralysis and death. Wide band white noise used in mammalian electroanaesthesia has been shown to reduce muscular tetany and was tested here for use with salmonids. Trials on rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*O. kisutch*) revealed that pulsed rectified wide band white noise (5-30 KHz), produced by a noise generator and a wide band amplifier, induced anaesthesia and reduced, but did not eliminate, damaging tetanic contractions.
Descriptors: anaesthesia, electrophysiology, *Oncorhynchus mykiss, Oncorhynchus kisutch*, INE, Canada, British Columbia
ASFA; Copyright © 2003, FAO

Mazur CF, Yesaki TY, Iwama GK (1991) **Improvements on the use of two alternatives to chemical anaesthesia for fish: Electroanaesthesia and CO_2 anaesthesia.** *Ices Council Meeting Papers., Ices, Copenhagen (Denmark).* 1 p.
There is an increasing need for the use of non-chemical anaesthesia for fish cultured for food as well as for wild fishery enhancement. This need arises from the potential impacts of chemicals on the environment, as well as the requirement to reduce chemical residues in food fish. This paper describes work that we have conducted in the refinement of the use of two alternatives to chemical anaesthetics. While both electroanaesthesia and CO_2 anaesthesia have been used for fishery and aquaculture purposes, both have drawbacks in their use in fish. Severe muscle tetany can result from electroanaesthesia, often resulting in spinal dislocation and muscle hemorrhaging. CO_2 anaesthesia causes irritation and a struggle response in exposed animals.
Descriptors: anaesthetics, bioaccumulation, tissues, environmental impact, aquaculture effluents, Oncorhynchus
ASFA; Copyright © 2003, FAO

McNeil FI, Crossman EJ (1979) **Fin clips in the evaluation of stocking programs for muskellunge, *Esox masquinongy*.** *Transactions of the American Fisheries Society.* 108(4):335-343
NAL Call No. 414.9 AM3
During laboratory and field experiments in Ontario, with hatchery muskellunge 90-235 mm in total length, total removal of a fin did not add to the immediate mortality caused by seining the fish from ponds. The use of an anesthetic during surgery (MS-222) did not affect subsequent survival of marked, stocked fish. Removal of any single paired fin was equally detrimental to short-term (3 months) survival. In contrast, over long periods (10 months) the loss of a pectoral fin was more detrimental than loss of a pelvic fin. Removal of both fins of a pair may cause higher mortality than the removal of one fin. Neither the fin removed nor the anesthetic significantly affected short-term or long-term growth. Within 1 year of marking regeneration of amputated fins was such that recognition of marked fish was difficult and the degree of difficulty increased with time. Estimates based on marked 2-year-old or older individuals could rsult in substantial underestimates of survival.
Descriptors: stocking (organisms), tagging mortality, *Esox masquinongy*, tagging, mortality causes, anaesthetics, survival, Esocidae, Pisces
ASFA; Copyright © 2003, FAO

McVicar AJ, Rankin JC (1983) **Renal function in unanaesthetized river lampreys (*Lampetra fluviatilis* L.): Effects of anaesthesia, temperature and environmental salinity.** *Journal of Experimental Biology.* 105:351-362

NAL Call No. 442.8 B77
Improved estimates of urine flow rates of lampreys in various salinites were obtained by the collection of urine for periods of up to 48 h from minimally-stressed, unanaesthetized fish, following catheterization of the urinogenital papilla. Urine flow rate in unanaesthetized fish was extremely sensitive to rapid (6°C/hour) changes in temperature and Q_{10} (6-16°C) was approximately 5. Urine flow rate decreased rapidly as the osmotic difference between the body fluids and environment approached zero, and the rate of flow in 30% seawater lampreys was only 7 multiplied by 6% that of freshwater fish. Present data compare favourably with data obtained previously from anaesthetized animals, indicating that renal function in lampreys is not significantly impaired by light MS222 anaesthesia.
Descriptors: urine, excretion, temperature effects, salinity effects, anesthetics, *Lampetra fluviatilis*
ASFA; Copyright © 2003, FAO

Mgbenka BO, Ejiofor EN (1998) **Effects of Extracts of Dried Leaves of Erythrophleum suaveolens as Anesthetics on Clariid Catfish.** *Journal of Applied Aquaculture.* 8(4):73-80
NAL Call No. SH135 J69
The effects of crude extract, pure extract, aqueous fraction of pure extract and lipid fraction of pure extract of air-dried leaves of *Erythrophleum suaveolens* as anesthetic on African sharptooth, *Clarias gariepinus*, and the African vundu catfish, *Heterobranchus longifilis*, fingerlings were studied. They were exposed to various doses of the extracts in tanks. The time for each fish to reach anesthesia were recorded. The two clariids were anesthetized in up to 3.5 g/L crude extract and recovered in the fresh water. Soaking the leaves for 24 hours or 48 hours produced no significant difference ($P > 0.05$) in the time to reach anesthesia for the African vundu catfish. These fingerlings reached anesthesia in significantly shorter time ($P < 0.05$) (24.5 minutes at 2.4 g/L concentration) in the pure unseparated extract than in the crude extract (70.5 minutes at 2.4 g/L concentration). All fingerlings exposed to 4 g/L extracts did not recover. Those exposed to less than 3.5 g/L of plant material were anesthetized and recovered only to die later within 24 hours. The time to reach anesthesia decreased with an increase in concentration of the plant extract. Of the two fractions, only the lipid fraction had anesthetizing effect on fish. It, however, took longer to produce the effect than the unseparated pure extract. The aqueous fraction of the pure extract and the control produced no observable anesthetic effects on the fish within 180 minutes. This suggests that the anesthetizing active ingredient resided in the lipid fraction but some factor in the aqueous layer was necessary to quicken its action. Similar results were got with the sharptooth catfish. Since the fingerlings died after recovering from anesthesia it was concluded that the safety margin of *E. suaveolens* for fingerlings was very narrow at the concentrations used. It is, therefore, not recommended for use on the fingerlings of the clariid catfishes.
Descriptors: fish culture, anaesthetics, fish handling, biological stress, *Erythrophleum suaveolens, Clarias gariepinus, Heterobranchus longifilis*
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Milton P, Dixon R.N (1980) **Further studies of the effects of the anaesthetic quinaldine on the physiology of the intertidal teleost *Blennius pholis*.** *Journal of the Marine Biological Association. UK.* 60(4):1043-1051
NAL Call No. 442.9 M331
Marked reductions in oxygen consumption were noted in high concentration of quinaldine (10 and 20 ppm), and both entry into anaesthesia and recovery from it were rapid. A period of enhanced oxygen consumption followed anaesthesia, except in the lowest concentration of

quinaldine (1 ppm). Experiments conducted over a 4 h period with three different salinities (100, 30 and 10% sea water) indicated that, under the influence of 10 and 20 ppm quinaldine solutions, the fish more resembled an osmoconformer than an osmoregulator. During anaesthesia, water was lost osmotically in 100% sea water, and gained in the more dilute salinities, although it was possible that some osmotic regulation continued. Physiological measurements indicated that quinaldine is suitable for the capture and marking of fish; for surgical procedures it should be mixed with another anaesthetic, for example MS-222, due to the retention of a response to vibratory stimuli.
Descriptors: anaesthetics, oxygen consumption, *Blennius pholis*, acclimatization, intertidal environment, Blenniidae, Pisces
ASFA; Copyright © 2003, FAO

Mishra BK, Kumar D, Mishra R (1983) **Observations on the use of carbonic acid anaesthesia in fish fry transport.** *Aquaculture.* 32(3-4):405-408
NAL Call No. SH1A6
Observations are reported on the use of carbonic acid anaesthesia in fish fry transport. The results indicate that live fry of *Labeo rohita* (Ham.) could be kept safely (with 95% survival) in the transport medium under a dose of 500 ppm of carbonic acid for as long as 215 h. Controls survived for only < 106 h.
Descriptors: stocking (organisms), fish larvae, anaesthetics, carbonic acid, survival, *Labeo rohita*
ASFA; Copyright © 2003, FAO

Morales AE, Garcia-Rejon L, Higuera Mde la, Billard R, Pauw Ndec (1989) **Use of anaesthesia in situ for handling stress suppression in rainbow trout.** *Aquaculture Europe '89. Short Communications and Abstracts of Review Papers, Films/Slideshows and Poster Papers, Presented at the International Aquaculture Conference Held in Bordeaux, France, 2-4 October, 1989., 1989, Special Publication, European Aquaculture Society.* 10:173-174
NAL Call No. SH138.S64
The findings are presented of experiments conducted to determine the effects of in situ anaesthetization of cultured rainbow trout (*Oncorhynchus mykiss*) on suppressing stress caused by routine laboratory handling of the fish. It is suggested that anaesthetics may be used to avoid stress responses under laboratory conditions, but not in fish farms.
Descriptors: laboratory culture, fish handling, biological stress, anaesthesia, *Oncorhynchus mykiss,* fish culture
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Munday PL, Wilson SK (1997) **Comparative efficacy of clove oil and other chemicals in anaesthetization of *Pomacentrus amboinensis*, a coral reef fish.** *Journal of Fish Biology.* 51(5):931-938
NAL Call No. QL614 J68
The efficacy of quinaldine, benzocaine, MS-222, 2-phenoxyethanol and clove oil was compared for anaesthetizing settlement stage Pomacentrus amboinensis, a frequently studied coral reef fish. Induction to anaesthesia, behaviour during anaesthesia, recovery times and survival rates of fish treated with the different chemicals were compared. Clove oil was only marginally less effective than quinaldine and was more effective than other chemicals tested, except at high concentrations. In addition, fish exposed to clove oil exhibited a much calmer induction to anaesthesia than fish exposed to quinaldine. Therefore, clove oil may be an effective alternative to quinaldine as a fish anaesthetic. Recovery time after anaesthesia with

clove oil was two to three times longer than recovery from other chemicals, a desirable characteristic for use in field studies. Survival rates were excellent for all chemicals.
Descriptors: anaesthesia, marine fish, coral reefs, survival, developmental stages, anesthetics, *Pomacentrus amboinensis*, clove oil, quinaldine, Pallid damselfish

Muzinic R (1970) **On the use of anaesthetics in the transportation of sardines.** *Studies and Reviews of the General Fisheries Council of the Mediterranean.* 47:1-23
The rate of mortality of non-selected sardines, exposed to 1:150,000 conc of tricaine methane sulfonate in open-system experiments increased rapidly and after 2 hr, by far exceeded 50 per cent. The mortality rate of the fish slowed down considerably when the sardines were transferred to a fresh anaesthetic solution at 30 min intervals using a conc of 1:150,000 tricaine methane sulfonate or compressed air when the temps were 20.8°C and 21.7°C; transferring the sardines had the same effect on their mortality using chloral hydrate at both 1:1,000 and 1:3,000 concs and the effect was even more notable at the latter concs. Similar procedure may be applied in transporting sardines, especially from distant localities for tagging and other experimental work. It is possible that some changes in the composition of the anaesthetic solution during the initial phase of transportation may be useful. With changes in the anaesthetic solution being made at 30-min intervals, lower concs of chloral hydrate were more advantageous. In standard anaesthesia experiments however, this was not so. In standard anaesthesia experiments with chloral hydrate, a rapid increase in mortality occurred at a decline of the O_2 conc to a point below 2 cc/1. In open system standard anaesthesia experiments using 1:150,000 tricaine methane sulfonate conc, the last sardine died at a temp ranging from 20.3°C to 22.8°C and at a mean final O_2 value of 0.56 + or - 0.46 cc/1. Chloral hydrate at 1:3,000 and 1:5,000 concs (and perhaps even lower ones) may replace tricaine methane sulfonate in transporting the sardines. The delicate state of the fish was shown by a marked variability of the mortality course within all the series of anaesthesia experiments and by a rather high mean final oxygen value and its great variation.

Descriptors: anesthetic, anesthesia, tranport, sardines, chloral hydrate

Nilsen H, Lillehaug A, Taksdal T, Nordmo R (1992) **Toxicity of intraperitoneally injected formalin in Atlantic salmon, *Salmo salar* L.** *Journal of Fish Diseases.* 15(4):323-329
NAL Call No. SH171 A1J68
Atlantic salmon, *Salmo salar* L., parr were injected intraperitoneally with different quantities of formalin (37% formaldehyde) following anaesthetization with either benzocaine (ethyl-p-aminobenzoate) or chlorbutol (1,1,1,-trichloro-2-methyl-2-propanol). The LD_{50} for injected formaldehyde was found to be approximately 50 mg/kg body weight. The type and concentration of the anaesthetic used did not influence mortality rates. Formalin is commonly used to inactivate microorganisms in vaccines. Doses of formalin which were shown to be toxic in this study are close to those which may be injected into fish as a component of various vaccines against bacterial fish diseases. Hence, the formalin content in fish vaccines may well cause mortality under certain conditions.
Descriptors: fish diseases, bacterial diseases, disease control, vaccination, fish culture, anaesthesia, *Salmo salar*, Atlantic salmon

Obradovic J (1986) **Effects of anaesthetics (halothane and MS-222) on crayfish, *Astacus astacus*.** *Aquaculture* 52(3):213-217
NAL Call No. SH1A6
The effects of an anaesthetic dispersive in air and usually applied in mammals (halothane, Hoechst) and of an anaesthetic soluble in water and applied in fish (MS-222, Sandoz) were investigated in experiments with crayfish, *A. astacus* (L.). Halothane was used at concentrations of 0.01, 0.06, 0.12, 0.5 and 1.0 vol. %, while MS-222 was applied in two concentrations, dissolved in the ratios of 1:1000 and 1:10,000. Halothane was most effective at 0.5 vol. %. The concentrations of MS-222 which were applied in the authors experiments had almost no effect on the crayfish.
Descriptors: crayfish culture, aquaculture techniques, anaesthetics, *Astacus astacus*, Halothane, MS-222

Oikawa S, Takeda T, Itazawa Y (1994) **Scale effects of MS-222 on a marine teleost, porgy *Pagrus major*.** *Aquaculture* 121(4):369-379
NAL Call No. SH1A6
The relationship between the effective concentration C_E of the anaesthetic MS-222 and body mass was examined at 20°C in porgy ranging in size from 0.00022 g (just after hatching) to 320 g (595 days old). C_E was defined as the concentration which required 3-5 min to induce deep anaesthesia in the fish. Values of C_E (ppm) increased monophasically with increasing body mass (W in g), following the equation C_E = 79W super(0.0549) (N = 30, r = 0.936 between log C_E and log W). The recovery ratio (R sub(R) in %), i.e., the number of fish as a percentage which recovered after being placed back in ordinary seawater, varied depending on the developmental stage. R sub(R) was lowest in fish of around 0.01 g (30 days old), corresponding to the transitional stage between the post-larval and juvenile stage, whereas it was highest (nearly 100%) in the pro-larval stage (about 0.00023 g in size and 0-6 days of age).
Descriptors: anesthetics, body size, recovery, fish culture, *Pagrus major*, anaesthesia, MS-222

Olsen YA, Einarsdottir IE, Nilssen KJ (1995) **Metomidate anaesthesia in Atlantic salmon, *Salmo salar*, prevents plasma cortisol increase during stress.** *Aquaculture* 134(1-2):155-168
NAL Call No. SH1A6
Atlantic salmon (*S. salar*) parr (58 g) in fresh water at 5.0°C and adult salmon (1130 g) in sea water at 7.7°C were exposed to water containing different concentrations of metomidate in the range 1 to 10 mg/l. Metomidate was efficacious in inducing anaesthesia (hypnosis), and efficacy increased with concentration over the interval tested. The anaesthetic was more potent in the adult salmon acclimated to sea water than in freshwater parr. Metomidate at 3 mg/l or higher completely prevented any plasma cortisol increase after a handling stressor when stressor and anaesthetic were applied concomitantly. The lack of a cortisol response seemed to be due to a blockage at the level of the interrenal cell, since exogenous ACTH injected intraperitoneally did not produce a cortisol increase in metomidate-anaesthetized fish but did in those anaesthetized with MS-222. Blood lactate levels and haematocrit increased in fish during metomidate anaesthesia.
Descriptors: fish culture, marine aquaculture, freshwater aquaculture, biological stress, anaesthetics, haematology, anadromous species, *Salmo salar*

Ortun J, Esteban MA, Meseguer J (2002) **Effects of phenoxyethanol on the innate immune system of gilthead seabream (*Sparus aurata* L.) exposed to crowding stress.** *Veterinary Immunology and Immunopathology.* 89(1-2):29-36
NAL Call No. SF757.2.V38
Phenoxyethanol is routinely used in seabream aquaculture to minimise fish stress response despite the secondary negative effects which have been observed. In this study, two different doses (60 and 200 mu l/l) of phenoxyethanol, sedative and narcotic, were tested for their ability to reduce the stress caused in gilthead seabream (*Sparus aurata* L.) by crowding. Blood glucose and serum cortisol concentrations were measured as stress indicators. In order to study the effects of the treatment on the innate immune system of crowded specimens, two parameters of the innate immune response, serum complement activity and phagocytosis, were assessed. The results show that anaesthesia itself produced a stress response in the fish and affected the immune system, although the effects were greater with the narcotic dose. When the effects of anaesthesia on crowded fish were analysed, the results pointed to a slight reduction in stress as a result of the sedative dose of phenoxyethanol (lower increase in cortisol and lower reduction in phagocytosis). However, additive negative effects were seen in crowded fish when the narcotic dose of phenoxyethanol was used. Since the use of phenoxyethanol is a common practice in aquaculture, the significance of the results should be considered.
Descriptors: drugs, anaesthetics, biological stress, immunity, stocking density, fish culture, complement, phagocytosis, anesthesia, stress, glucose, hydrocortisone, aquaculture, fish immunity, phenoxyethanol, *Sparus aurata*, phenoxyethanol, gilthead seabream, crowding
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Ortuno J, Esteban MA, Meseguer J (2002) **Effects of four anaesthetics on the innate immune response of gilthead seabream (*Sparus aurata* L.).** *Fish & Shellfish Immunology.* 12(1):49-59
NAL Call No. QL638.97.F55
Anaesthesia may depress the immune system in mammals, but there is no available information on this topic in fish. In the present work, four anaesthetics that are used in aquaculture, MS222 (0.19 mm), benzocaine (0.21 mm), 2-phenoxyethanol (1.6 mm) and quinaldine sulphate (0.083 mm), were tested in order to observe their effects on the gilthead seabream (*Sparus aurata* L.) innate immune system. The results showed that the four anaesthetics produced increased blood glucose levels after an hour. In addition, benzocaine and 2-phenoxyethanol depressed complement activity and phagocytosis, while MS222 and quinaldine sulphate did not. Some anaesthesia is a common practice in aquaculture, the data obtained should be taken into account to avoid possible immunodepression in farmed fish. Copyright 2002 Academic Press
Descriptors: immune response, anesthetics, glucose, blood levels, phagocytosis, fish culture, cultured organisms, marine fish, fish physiology, disease resistance, husbandry diseases, immunity, haematology, anaesthesia, fish immunity, benzocaine, 2-phenoxyethanol, quinaldine sulfate, *Sparus aurata*, gilthead seabream, immunodepression, blood glucose levels, MS222
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Ortuno J, Esteban MA, Meseguer J (2002) **Lack of effect of combining different stressors on innate immune responses of seabream (*Sparus aurata* L.).** *Veterinary Immunology and Immunopathology.* 84(1-2):17-27
NAL Call No. SF757.2.V38
A complex stressful event, which commonly occurs in modern aquacultural practices, was

broken down into factors that were analysed both individually and jointly to assess their effect on two stress indicators (blood glucose and serum cortisol levels) and two activities of the innate immune response (serum complement and head-kidney leukocyte respiratory burst). For this, gilthead seabream (*Sparus aurata* L.) specimens were exposed to the following stressors: physical disturbance, crowding, anaesthesia with 2-phenoxyethanol and air exposure. At 0, 1, 2, 3 and 4 days post-stress, blood and serum samples were collected to measure glucose concentration and cortisol and complement levels, respectively. Head-kidney leukocytes were isolated and assayed to evaluate respiratory burst activity. The results show that physical disturbance, crowding and anaesthesia produced an occasional increase in glucose and cortisol concentrations. Crowding and anaesthesia induced a depression in complement activity, while hypoxia by air exposure caused a reduction in the respiratory burst. When all factors were jointly applied both humoral and cellular defenses were compromised and cortisol values remained high throughout the experimental period. Any long-term effects of this abnormal serum cortisol levels on the immune system remain unknown.
Descriptors: fish immunity, leukocytes, 2-phenoxyethanol, glucose, cortisol, immunity, biological stress, stocking density, anaesthesia, air exposure, defense mechanisms, handling, aquaculture techniques, *Sparus aurata*, gilthead seabream, cortisol
ASFA; Copyright © 2003, FAO

Osanz Castan E, Esteban Alonso J, del Nino Jesus A, Josa Serrano A, Espinosa Velazquez E (1993) **Study on quinaldine and 2-phenoxyethanol anaesthetics: Age and specie influence.** *Actas Del IV Congreso Nacional De Acuicultura., Centro De Investigaciones Marinas, Pontevedra (Spain)*. pp. 737-742
We have studied the 2-phenoxyethanol and quinaldine effect on adult and fry carp and the 2-phenoxyethanol effect on adult tench (*Tinca tinca*) and carp (*Cyprinus carpio*). We have used different temperatures (10°C in winter and 20°C in summer) and exposure time (1', 3', 5'. 7' and 10' minutes). Anaesthetic doses employed are: 0.1, 0.2, 0.35 and 0.5 ml/l in 2-phenoxyethanol and 0.0125, 0.0250, 0.0375 and 0.05 ml/l in quinaldine.
Descriptors: fish culture, anaesthesia, serological studies, blood, temperature, fry, *Tinca tinca, Cyprinus carpio*, 2-phenoxyethanol
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Peres G, Roche H, Skrzynski J (1989) **The importance of hematological modifications of a biochemical nature provoked by anesthesia in the fish.** *Bulletin de l'Academie Veterinaire de France*. 62(2):259-272
NAL Call No. 41.9 R24
Results of an experimentation using MS222 (Tricain Methane Sulfonate) in the case of the sea fish *Dicentrarchus labrax* and bibliographic data show that anaesthesia can induce more or less great modifications of the biochemical hematology in function of utilization. It may induce three kinds of consequence; a transient alteration of the sanitary state; an interaction with some experimental process which might be erroneous; an eventual incidence upon animal or human consumers which justifies precautions concerning alimentary utilization.
Descriptors: haematology, biochemical composition, human food, *Dicentrarchus labrax*, anaesthetics, MS222
ASFA; Copyright © 2003, FAO

Plumb JA, Schwedler TE, Limsuwan C (1983) **Experimental anesthesia of three species of freshwater fish with etomidate.** *Progressive Fish-Culturist.* 45(1):30-33

NAL Call No. 157.5 P94
Etomidate (ethyl-1-methylbenzyl-imidazole-5-carboxylate) is an experimental non-barbiturate hypnotic agent used intravenously for anesthetic induction in humans and domestic or laboratory mammals. Three species of fish (golden shiners, *Notemigonus crysoleucas* ; striped bass, *Morone saxatilis* ; and channel catfish, *Ictalurus punctatus*) were anesthetized for up to 96 h with various concentrations of etomidate. Mortality during the 48-h recovery period after anesthesia was low in all species of fish. Two-year-old channel catfish withstood from 0.4 to 3.6 mg/L for 80 min with low mortality at the higher concentrations, but anesthesia was induced at 0.8 to 1.2 mg/L. Etomidate compared favorably to quinaldine and tricaine methanesulfonate under the conditions tested.
Descriptors: anesthetics, experimental research, *Notemigonus crysoleucas, Morone saxatilis, Ictalurus punctatus*, etomidate

Post G (1979) **Carbonic acid anesthesia for aquatic organisms.** *Progressive Fish-Culturist.* 41(3):142-144
NAL Call No. 157.5 P94
Carbonic acid in water is a safe, inexpensive, effective, convenient, and easily obtainable anesthetic for fish and other aquatic organisms. Carbonic acid is not a controlled substance in the United States, and may not require US Food and Drug Administration registration. Baths containing 150 to 600 mg/l carbonic acid (HSUB-2 COSUB-3) will anaesthetize fish; lower concentrations anaesthetize more slowly and less deeply, high concentrations act more rapidly and with greater sedation. The length of time that fish or aquatic organisms can safely be held in anaesthetizing baths depends on the carbonic acid concentration, and is longer at low concentrations than at high concentrations. A convenient procedure for developing known concentrations of carbonic acid in anaesthetizing baths involves adding equal volumes of 6.75% (wt/vol) sodium bicarbonate solution and 3.95% (wt/vol) of concentrated (97-98%) sulphuric acid solution to a known volume of water.
Descriptors: anaesthetics, carbonic acid

Prihoda J (1979) **Experience with use of propoxatein anaesthesia and transport of salmonids in Slovakian fishery union centres.** *Biologizace a Chemizace Zivoicisne Vyroby-Veterinaria.* 15(3):283-288
NAL Call No. SF1.B5
Following previous tests, anaesthetic Propoxat of Janssen Co. was introduced into the fishery practice in all the centres of Slovakian Fishery Union during the spawning period of breeding fishes. To facilitate the application, 1% solution dosed 1 ml per 5L water (solution 1:500,000) is used in practice. With view to the size of the medicated basin, 30 to 50 fish are narcotized and after 2.5 min they are transferred to a canvas cradle and spawned, while another batch of fish is put into the basin. The solution is changed after 8 bathes. In the course of the five-year usage no mortality was recorded owing to anaesthesia. In testing an appropriate Propoxate concentration and the method of transporting fish, hermatically closed polyethylene tanks containing 20 l of transporting solution with Propoxate diluted 1:8,000,000 and filled with 16 kg of fish and 20 l of oxygen approved their suitability for this purpose. No negative changes ere found after a two hour transport, while 850 heads out of 5,000 control rainbow trouts died during the classical transport in aerated water.
Descriptors: fish culture, spawning, anasthetics, anaesthesia, induced breeding, Salmonidae, Czechoslovakia

Prince A, Powell C (2000) **Clove Oil as an Anesthetic for Invasive Field Procedures on Adult Rainbow Trout.** *North American Journal of Fisheries Management.* 20(4):1029-1032
NAL Call No. SH219.N66
Clove oil has recently been proposed as an appropriate anesthetic for researchers to use on food fish; however, its use in invasive procedures has not yet been reported for salmonids. In a telemetric investigation, we found the concentration of clove oil required for invasive procedures to be 30 mg/L, which is 75% less than previously suggested dosages to achieve and maintain a level of deep anesthesia in adult rainbow trout *Oncorhynchus mykiss* (N = 20). Mean time (± SE) to achieve level 4 anesthesia was 3.7 ± 0.9 min. Average exposure time to the anesthetic (surgery time) was 5.8 ± 0.2 min, and average recovery time (the time required to regain equilibrium and full swimming mobility) was 4.9 ± 1.0 min. Lengthy recovery times (up to 18 min) were observed; such times are typically reported for clove oil. Field investigators should conduct preliminary trials to determine the appropriate concentration of clove oil for their conditions, particularly if the application requires more than 5 min of exposure to the anesthetic.
Descriptors: anaesthetics, bioassays, adults, *Oncorhynchus mykiss*, clove oil, Rainbow trout
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Puceat M, Garin D, Freminet A (1989) **Inhibitory effect of anaesthesia with 2-phenoxyethanol as compared to MS222 on glucose release in isolated hepatocytes from rainbow trout (*Salmo gairdneri*).** *Comparative Biochemistry and Physiology.* 94A(2):221-224
NAL Call No. QP1 C6
Glucose production by freshly isolated hepatocytes from rainbow trout was studied after anaesthesia of the animals with 2-phenoxy ethanol or tricaine methanesulphonate. At the end of the procedure hepatic contents of glycogen, glucose, lactate, ATP, ADP, AMP, were not significantly different between the two treatments.
Descriptors: liver, cells, inhibitors, anaesthesia, *Salmo gairdneri*, fish culture, fish physiology, biochemical phenomena, glucose, hepatocytes, 2-phenoxyethanol, modulation, release
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Rivera Lopez H, Orbe Mendoza A, Ross LG (1991) **Use of xylocaine, potentiated with sodium bicarbonate, as an anaesthetic for fry and juveniles of acumara, *Algansea lacustris* Steindachner 1895, from Lake Patzcuaro, Michoacan, Mexico.** *Aquaculture and Fisheries Management.* 22(1):15-18
The effectiveness of xylocaine anaesthesia, potentiated with sodium bicarbonate, was investigated on the altiplano cyprinid, *Algansea lacustris* Steindachner. The drug gave excellent sedation, handling and recovery and was effective at doses between 50 and 300 mg/l in 1 g/l sodium bicarbonate.
Descriptors: fish handling, anaesthesia, aquaculture techniques, *Algansea lacustris*, fry, juveniles, Mexico, Michoacan, Patzcuaro Lake, drugs, fish culture, xylocaine, sodium bicarbonate
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Ross LG, Ross B (1999) **Anaesthetic and sedative techniques for aquatic animals.** *Blackwell Science, Oxford (UK).* 76 pp.
NAL Call No. SH156.9 R67 1999
The handling of fish and other aquatic animals in an out of their natural environment is always difficult. Their struggling has strong effects on their physiology and behaviour and the animals can easily be damaged. Anaesthesia and sedation are therefore essential

techniques in fisheries management and aquaculture to minimize stress and physical damage caused by crowding, capture, handling and release. This new text fulfils a proven need for an illustrated, practical guide for workers in aquaculture and fisheries research and management. Based on first-hand experience, the text covers fish, amphibian and reptiles and includes a glossary of drugs, an explanation of major technical terms and an index for ease of reference.
Descriptors: disease control, aquaculture, fish diseases, anaesthetics, fish handling

Ross LG, Ross B (1984) **Anaesthetic and sedative techniques for fish.** *Institute of Aquaculture, Stirling (Uk),* 42 pp.

The purpose of this handbook is to draw together the available information on sedation and anaesthesia of fishes. Both temperate and tropical freshwater species are considered as well as sedation in sea water. While sedation is a a routine and essentially simple procedure it can also be mismanaged. The overall intention is therefore to produce an illustrated, practical guide for workers both in aquaculture and in research.
Descriptors: anaesthesia, anesthetics, fish, Pisces

Ross RM, Backman TWH, Bennett RM (1993) **Evaluation of the anesthetic metomidate for the handling and transport of juvenile American shad.** *Progressive Fish-Culturist* 55(4):236-243

Juvenile American shad (*Alosa sapidissima*) were exposed to three levels of metomidate (0.0 = control, 0.5, and 1.0 mg/L) and three types of sedation or handling (none, sedation only, and handling after sedation) to determine the efficacy and safety of the drug for use in transport and handling of this species. Mean sedation times were 9 and 3 min, and mean recovery times were 6 and 7 min, respectively, for the 0.5- and 1.0-mg/L concentrations of metomidate. For fish exposed to 1.0 mg metomidate/L, normal swimming behavior was delayed as long as 4 h after fish were placed in drug-free water. Aggregating and parallel orientation behaviors, precursors of normal schooling, were significantly reduced at the highest drug level for 1 h, but not for 24 h, after recovery. No posttreatment difference in behavior was observed as a result of handling fish (removal from water, weighing, and measuring) under sedation. Long-term (50-d) survival was not affected by drug concentration in one experiment and was improved by use of the anesthetic in a second study. Metomidate appears to be useful and safe for the transportation and handling of juvenile American shad.
Descriptors: anaesthetics, handling, transport, juveniles, *Alosa sapidissima*, metomidate

Roubach R, De Carvalho Gomes L, Val AL (2001) **Safest level of tricaine methanesulfonate (MS-222) to induce anesthesia in juveniles of matrinxa, *Brycon cephalus*.** *Acta Amazonica.* 31(1):159-163

The use of MS-222 as an anesthetic for matrinxa juveniles was investigated. At dosage of 100 mg/L or lower fish did not achieve a complete anesthesia state. At 150 mg/L, MS-222 induced anesthesia within 36 seconds and recovered from a 10 minutes period of anesthesia within 5.2 min. Higher concentrations (200, 250 and 300 mg/L) anesthetized fish in lesser times, with the offset of mortality (16.7 and 33.3%) at the 200 and 300 mg/L MS-222 doses, respectively. The only significant differences observed in the hematological parameters, was

for the glucose values in fish anesthetized with 250 and 300 mg/L. From the results, the recommended dose of MS-222 for handling matrinxa juveniles is 150 mg/L.
Descriptors: anaesthetics, anaesthesia, juveniles, fish handling, *Brycon cephalus*, tricaine methanesulfonate
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Sado EK (1985) **Influence of the anaesthetic quinaldine on some tilapias.** *Aquaculture.* 46(1):55-62
NAL Call No. SH1A6
Three species of tilapia, *Sarotherodon melanotheron* and *Sarotherodon guincensis* from brackish water and *Oreochromis (Sarotherodon) noloticus* from fresh water, were exposed to different concentrations of the anaesthetic quinaldine to determine the safe level for handling and transportation of these species. Dosages of quinaldine for handling fish for experimental work and for transportation are given with the proviso that the anaesthetization is carried out under conditions of salinity and temperature which are suitable for acclimation.
Descriptors: anesthetics, live storage, *Sarotherodon melanotheron, Sarotherodon guincensis, Oreochromis niloticus*, environmental factors, quinaldine
ASFA; Copyright © 2003, FAO

Sandodden R, Finstad B, Iversen M (2001) **Transport stress in Atlantic salmon (*Salmo salar* L.): anaesthesia and recovery.** *Aquaculture Research.* 32(2):87-90
NAL Call No. SH1 F8
The effects of metomidate anaesthesia on levels of plasma cortisol, glucose, haematocrit and chloride in Atlantic salmon (*Salmo salar* L.), after a 2-h transport and during a 48-h recovery period were investigated. The use of metomidate anaesthesia during transport led to a reduced release of cortisol and significantly lower levels of plasma cortisol after a 48-h recovery period. Plasma glucose did not return to basal level after a 48-h recovery period, indicating that even longer reccvery may be needed for the fish to return to a pre-stress state. The results show that metomidate anaesthesia combined with a recovery period lessens the stress burden imposed by hauling and transport.
Descriptors: biological stress, anaesthesia, transportation, fish culture, fish physiology, serological studies, *Salmo salar*, Atlantic salmon
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Sayer MDJ, Cameron KS, Wilkinson G (1994) **Fish species found in the rocky sublittoral during winter months as revealed by the underwater application of the anaesthetic quinaldine.** *Journal of Fish Biology.* 44(2):351-353
NAL Call No. QL614 J68
Using the anaesthetic quinaldine applied underwater, fish species not normally observed in the Scottish rocky sublittoral during the winter months have been recorded, and are listed.
Descriptors: check lists, distribution records, marine fish, rocky shores, littoral zone, winter, ANE, British Isles, Scotland, anaesthetics, Pisces, methodology, species composition, coastal waters
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Schramm HL Jr, Black DJ (1984) **Anesthesia and surgical procedures for implanting radio transmitters into grass carp.** *Progressive Fish-Culturist.* 46(3):185-190
NAL Call No. 157.5 P94
The authors investigated anesthetic and surgical procedures useful for implanting radio

transmitters into the body cavity of 1.6-3.7 kg (3.5-8.2 lb) grass carp (*Ctenopharyngodon idella*). Quinaldine was an effective anesthetic for fish at water temperatures < 26°C (79 °F); however, it was lethal at concentration necessary to induce and maintain sufficient anesthesia for surgery at water temperatures > 29 °C (84 °F). MS-222 was a suitable anesthetic at all water temperatures. Implantation of radio tags through midventral incisions was preferable to implantation through lateral incisions, because there was no danger of puncturing the ovaries of female fish and the operation was more easily performed. Surgical procedures are presented.
Descriptors: *Ctenopharyngodon idella*, biotelemetry, activity patterns, local movements, anaesthesia, surgical implantation

Sekizawa Y, Umemura K, Shimura M, Suzuki A, Kikuchi T (1975) **Residue analyses on 2-amino-4-phenylthiazole, a piscine anesthetic, in fishes 1. A model radiotracer experiment with medaka.** *Bulletin of the Japanese Society of Scientific Fisheries.* 41(4):449-458
NAL Call No. 414.9 J274
Residue analyses on Super(3)H.2-amino-4-phenylthiazole were performed in medaka (killifish, *Oryzias latipes*).This fish was selected because of its ready application in radiotracer experiments and its marked and unique ability to detoxify the piscine anesthetic, 2-amino-4-phenylthiazole to its N-hexuronyl conjugate. The absorption/excretion balance showed an average 102% recovery indicating that the excretion treatment effectively exhausted the residue from medaka, The 1/2 life for retention of this compd and its N-hexuronyl conjugate in the body of the fish was approx 12-15 hr. The fates of both the anesthetic and its N-hexuronyl conjugate in the fish body were determined separately in a time course survey using TLC radiography. plots for the excretion of the major product, i.e. the conjugate, and the anesthetic per se produced 2 crossing exponential curves suggesting that there are 2 compartmentations of both the conjugate and the anesthetic per se in medaka. A mathematical simulation of the residue was also described.
Descriptors: anaesthesia, bioaccumulation, chemical analysis, metabolism, *Oryzias latipes*

Sharma SK, Sharma LL (1996) **Effect of chloral hydrate on metabolic rate of *Labeo rohita* (Ham.) and *Poecilia reticulata* (Peters).** *Journal of the Indian Fisheries Association.* 26:121-125
Comparative impact of chloral hydrate anaesthesia on the metabolic rate of Indian major carp *Labeo rohita* and larvivorous fish *Poecilia reticulata* was assessed. Observation on the oxygen consumption rate (OCR) revealed that in common guppies OCR was substantially low (1.105 and 1.097 mg/g/hr) at 0.1 and 0.25 g/l concentrations of chloral hydrate as against OCR of 1.487 mg/g/hr in the control. Fry *L. rohita* in group showed lower metabolic rates in the control as well as treated conditions as compared to the individuals of this fish. This may be due to sympathetic psychophysiological reflex of grouped fish. Higher dose of chloral hydrate (0.25 g/l) also caused higher OCR probably due to distress. Application of chloral hydrate also favoured lesser release of metabolic wastes (ammonia and carbon dioxide). There was significant positive correlation between time and oxygen consumption, whereas, for time and OCR this relationship was negative. Regression of chloral hydrate doses for OCR and time has also been calculated.
Descriptors: anaesthetics, oxygen consumption, freshwater fish, *Labeo rohita, Poecilia reticulata*, India, metabolism, correlation, regression analysis

Shimura M, Sekizawa Y (1975) **Residue analyses on 2-amino-4-phenylthiazole, a piscine anesthetic, in fishes 2. Resolution on chemical structure of the metabolite in medaka.** *Bulletin of the Japanese Society of Scientific Fisheries.* 41(5):529-534
NAL Call No. 414.9 J274
The metabolite of 2-amino-4-phenylthiazole in medaka (killifish, *Oryzias latipes*) was isolated as white crystals from environmental water in which the fish were treated with the anesthetic. Acid hydrolysis of the metabolite gave crystallize 2-amino-4-phenylthiazole and a spot on TLC coinciding with that of D-glucuronic acid. Gas chromatography of the metabolite after methanolysis and trimethylsilylation gave peaks coinciding with those of authentic D-glucuronic acid and 2-amino-4- phenylthiazole. The colour reaction of the metabolite suggested that the conjugation site of these 2 constituents was the primary amino group of the thiazole ring and the aldehyde group of glucuronic acid. Reaction of D-glucuronic acid and 2-amino-4-pheylthiazole in 50% acetone under reflux resulted in the formation of a product identical with the naturally occurring metabolite. The NMR spectrum of the metabolite in D Sub(2)O revealed that the structure of the sugar moiety was {beta}-D-glucopyranosiduronic acid. Thus it was concluded that the chemical structure of the metabolite in medaka should be 2-amino-4- phenylthiazole 2-N-{beta}-D-glucopyranosiduronic acid.
Descriptors: anaesthesia, metabolites, *Oryzias latipes*, chemical structure
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Sijm DTHM, Bol J, Seinen W, Opperhuizen A. (1993) **Ethyl m-aminobenzoate methanesulfonate dependent and carrier dependent pharmacokinetics of extremely lipophilic compounds in rainbow trout.** *Archives of Environmental Contamination and Toxicology.* 25(1):102-109
NAL Call No. TD172 A7
Significant differences were found in both uptake and pharmacokinetics in fish (*Oncorhynchus mykiss*) when six lipophilic compounds were dosed by gavage in either an oil or an gelatin carrier. Pharmacokinetics were also different when fish were anaesthetized with ethyl m-aminobenzoate methanesulfonate (MS-222) before dosing. The highest uptake percentages, uptake rates and concentrations of the compounds were found in the fish which were given the gelatin carrier only. MS-222 decreased the uptake of the compounds. Absorption of the compounds from oil was lower than from gelatin. In addition, absorption from oil continued for 21 d, which lasted longer than from gelatin.
Descriptors: pharmacokinetics, lipids, chemicals, bioaccumulation, absorption, oil, gelatin, liver, diets, *Oncorhynchus mykiss*, tricaine, oils, chemical kinetics, anaesthesia, correlation, absorption coefficient, chlorinated hydrocarbons, freshwater fish, anadromous species
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Siwicki A (1984) **New anaesthetic for fish.** *Aquaculture.* 38(2):171-176
NAL Call No. SH1A6
Anaesthetics are needed when handling fish, especially during tagging. However, most anaesthetics applied at present have a strong toxic effect on fish. For this reason it is only permissible to keep fish anaesthetized for a short time. A new anaesthetic "Propanidid" has been successfully tested which allows fish to be anaesthetized for up to 1 h. It can be applied as an intraperitoneal injection and as a bath solution. The required disappearance of sense perception and motor reflexes in the fish can be obtained in 2-4 min. Low toxicity of the pharmacological preparation has been proved according to a full set of the clinical, toxicological, haematological and biochemical criteria. Clinical tests were carried out with

salmonids mainly. The new compound belongs to a group of anaesthetics which are used in human medicine; thus, it is considered to be harmless for man as an operator and a consumer.
Descriptors: fish culture, aquaculture techniques, anesthetics, fish handling, Propanidid
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Siwicki A, Jeney Z (1986) **Surgical intervention in wels (*Silurus glanis* L.) during artificial propagation.** *Aquacultura hungarica. Szarvas [Aquacult. Hung.].* 5:55-58
NAL Call No. SH101 H8A68
Experience obtained during surgical intervention in artificial propagation of wels (*Silurus glanis* L.) is described. Favourable results were obtained when operating the lateral part of fish at a height of testes applying sterile surgical line thread in 0.5 cm sutures. During operations the anaesthetic Propanidid (Polfa, Poland) was successfully applied.
Descriptors: fish culture, induced breeding, anaesthesia, *Silurus glanis*, testes, surgery
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Smit GL, Hattingh J (1979) **Anaesthetic potency of MS 222 and neutralized MS 222 as studied in three freshwater fish species.** *Comparative Biochemistry and Physiology.* 62C(2):237-241
NAL Call No. QP901 C6
The effects of several different concentrations of MS 222 and neutralized MS 222 on aquarium water quality and anesthetic potency were investigated in *Cyprinus carpio*, *Sarotherodon mossambicus* and *Salmo gairdneri*. MS 222 caused a decrease in pH and bicarbonate alkalinity with a corresponding increase in pCO_2 of the aquarium water. Conductivity was also increased. Neutralized MS 222 prevented, apart from an increase in conductivity, such effects. Increasing the concentration of MS 222 in the aquarium water resulted in a decrease in anaesthetic induction times in all three fish species studied. Neutralized MS 222 induced anaesthesia reduced induction times further and also increased recovery times from anaesthesia. The amount of free MS 222 in the blood of the three fish species studied did not differ significantly from each other per MS 222 concentration employed and showed an increase with increased concentrations of MS 222. With neutralized MS 222 the concentration of free MS 222 in the blood of the three fish species was generally higher. Neutralized MS 222 thus resulted in a deeper, more consistent anaesthesia, thereby indicating a safer, more effective, longer-acting anaesthetic.
Descriptors: anaesthetics, Pisces, aquaria, *Cyprinus carpio*, *Sarotherodon mossambicus*, *Salmo gairdneri*, water quality
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Smit GL, Hattingh J, Burger AP (1979) **Haematological assessment of the effects of the anaesthetic MS 222 in natural and neutralized form in three freshwater fish species: haemoglobin electrophoresis, ATP levels and corpuscular fragility curves.** *Journal of Fish Biology.* 15(6):655-663
NAL Call No. QL614 J68
The effects of MS 222 and neutralized MS 222 anaesthesia on haemoglobin electrophoresis, erythrocyte adenosine triphosphate (ATP) levels and corpuscular fragility curves were studied in *Cyprinus carpio*, *Sarotherodon mossambicus* and *Salmo gairdneri*. Haemoglobin electrophoresis showed no significant intra-species differences in the percentage composition of the various fractions for any concentration of MS 222 and neutralized MS 222 used. Significant interspecies differences were, however, still observed. ATP levels showed intra- and interspecies differences ascribed to the response of the fish species to MS 222-induced stress and not to actual changes in erythrocyte ATP concentrations. Differences were also

observed in corpuscular fragility curves for all three species when using MS 222 or neutralized MS 222 compared to curves obtained without the use of the anaesthetic, but the mechanisms involved are not clear.
Descriptors: haematology, anaesthetics, erythrocytes, haemoglobin, *Cyprinus carpio*, *Sarotherodon mossambicus*, *Salmo gairdneri*, Cyprinidae, Salmonidae, Cichlidae, Pisces, ATP, ethyl <m->aminobenzoate, effects on, fish
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Smit GL, Hattingh J, Burger AP (1979) **Haematological assessment of the effects of the anaesthetic MS 222 in natural and neutralized form in three freshwater fish species: interspecies differences.** *Journal of Fish Biology.* 15(6):633-643
NAL Call No. QL614 J68
Interspecies haematological differences to MS 222 and neutralized MS 222 anaesthesia were investigated in *Sarotherodon mossambicus*, *Cyprinus carpio* and *Salmo gairdneri* acclimated under identical laboratory conditions. Anaesthesia with MS 222 resulted in a 'chemical stress' in all fish, as was evident from changes in the haematological profiles of the animals. Such species variations in the haematology persisted throughout the whole experiment protocol which employed different concentrations of the anaesthetic. The use of neutralized MS 222, whereby aquarium water quality remained unchanged, improved the haematological profile. Possible reasons for the interspecies differences observed are discussed.
Descriptors: haematology, anaesthetics, *Cyprinus carpio, Salmo gairdneri, Sarotherodon mossambicus*, Cyprinidae, Salmonidae, Cichlidae, Pisces, fish culture, ethyl m-aminobenzoate, effects on, sedatives, local anaesthetics
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Smit GL, Hattingh J, Burger AP (1979) **Haematological assessment of the effects of the anaesthetic MS 222 in natural and neutralized form in three freshwater fish species: intraspecies differences.** *Journal of Fish Biology.* 15(6):645-653
NAL Call No. QL614 J68
The effects of different concentrations of natural MS 222 and neutralized MS 222 were studied on the haematology of *Cyprinus carpio*, *Sarotherodon mossambicus* and *Salmo gairdneri*. As judged from the results, MS 222, which is acidic in nature, produced haemoconcentration effects in all species studied, being least in the trout followed by carp and Sarotherodon mossambicus. These differences are ascribed to acid-base regulatory functions and metabolic activities of the fish species investigated. The use of neutralized MS 222 improved the haematological profiles markedly and resulted in stabilization of acid-base parameters and red blood cells sizes and numbers. Haemoconcentration effects, however, still persisted. Trout were found to be more susceptible to the stress of MS 222 anaesthesia than carp and *Sarotherodon mossambicus*.
Descriptors: haematology, anaesthetics, *Sarotherodon mossambicus*, *Salmo gairdneri*, *Cyprinus carpio*, Cichlidae, Salmonidae, Cyprinidae, ethyl-m-aminobenzoate, neutralization, effects on, sedatives, local anaesthetics
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Smit GL, Schoonbee HJ, Barham WT (1979) **Some effects of the anaesthetic MS 222 on fresh water.** *South African Journal of Science* 73(11):351-352
NAL Call No. 515 SO84
Previous research has shown that MS 222 (tricaine methanesulphonate) added to water to produce anaesthesia in fish has a variable effect. The possibility that this may be caused by

differences in mineral content was investigated in the study reported. MS 222 was added to tapwater, deionized water, distilled water, borehole water and water from a dolomite spring and the pH, pCO_2, pO_2, orthophosphate content and conductivity was measured at various times up to 1 1/2 h. Results show that because of the changes in pH and conductivity induced in deionized and distilled water, these should not be used in laboratory experiments in the presence of MS 222. The changes, which occur within 2 minutes and continue for at least 30 minutes, could significantly affect the physiology of target fish. However, water with a good buffering ability, such as that from the dolomite spring, was relatively unaffected by MS 222. It is concluded that water with a high mineral content is best suited for the laboratory use of MS 222.
Descriptors: anaesthetics, environmental effects, Pisces, physiology

Smith DA, Smith SA, Holladay SD (1999) **Effect of previous exposure to tricaine methanesulfonate on time to anesthesia in hybrid tilapias.** *Journal of Aquatic Animal Health.* 11(2):183-186
NAL Call No. SH171.J68
Tricaine methanesulfonate (MS-222) is one of the most broadly used anesthetic and tranquilizing agents for poikilotherms, and it is currently the only anesthetic approved for use with food fish. To test the preliminary observation in tilapias *Oreochromis sp.* previously exposed to anesthetizing doses of MS-222 that time to anesthesia was shorter on subsequent exposures, hybrids of white tilapias (Nile tilapia *O. niloticus* x blue tilapia *O. aureus*) crossed with Mozambique tilapias *O. mossambicus* were anesthetized with MS-222. Time to anesthesia was recorded at this initial drug exposure and weekly for 6 weeks after initial exposure. Fish previously exposed to MS-222 did not display a significantly reduced time to anesthesia on the second exposure but did display significant reductions at the third exposure and thereafter. These preliminary results suggest that tilapias do not respond to MS-222 with the typical enzyme induction-mediated tolerance reaction commonly seen with anesthetic chemicals in mammals.
Descriptors: hybrids, anaesthesia, tolerance, enzymes, oreochromis, tricaine methanesulfonate

Smith MFL (1992) **Capture and transportation of elasmobranchs, with emphasis on the grey nurse shark (*Carcharias taurus*).** *Sharks: Biology And Fisheries., Australian Journal of Marine and Freshwater Research.* 43(1):325-343
NAL Call No. QL638.9 I57 1991
Physiological changes manifest during the capture and transportation of elasmobranchs are discussed. Reference is made to the general adaptation syndrome and to mechanisms of change in concentrations of blood glucose, blood acid and serum electrolyte. Methods of alleviating these profound changes are suggested, with emphasis on the capture and transportation of the grey nurse shark (*Carcharias taurus*). The hoop method is shown to be a convenient technique for capturing wild grey sharks, and a combination of ketamine hydrochloride and xylazine hydrochloride, with antagonism by yohimbine hydrochloride, is found to provide an acceptable regime of transportation anesthesia. Intravenous administration of sodium bicarbonate and glucose as well as oxygenation of water in the life-support system are shown to be valuable methods of alleviating stress-induced blood acidosis.
Descriptors: captivity, transportation, *Carcharias taurus*, biological stress, fish culture, fish handling, anaesthesia

Soivio A, Hughes GM (1978) **Circulatory changes in secondary lamellae of *Salmo gairdneri* gills in hypoxia and anaesthesia.** *Annales Zoologici Fennici.* 15(3):221-225
NAL Call No. 410 AN712
The secondary lamellae of rainbow trout gills from hypoxic and anaesthetized fish were analysed stereologically for the blood volume and haematocrit value. In hypoxia the gills undergo vasodilatation, which is accompanied by slight haemodilution. When oxygen is available the situation changes; there is a tendency to vasoconstriction, but a continuing decrease in the haematocrit value. In anaesthesia the vasodilatation seen in the secondary lamellae is combined with haemoconcentration. During recovery vasodilatation continues, while the haematocrit value falls to below that of controls. Explanations for these observations are offered in terms of the circulation through the gills.
Descriptors: respiratory organs, blood circulation, *Salmo gairdneri*, physiology, anaesthesia, hypoxia, blood
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Soivio A, Nyholm K, Huhti M (1977) **Effects of anaesthesia with MS 222, neutralized MS 222 and benzocaine on the blood constituents of rainbow trout, *Salmo gairdneri*.** *Journal of Fish Biology.* 10(1):91-101
NAL Call No. QL614 J68
Rainbow trout (*S.gairdneri* Richardson) were subjected to 15 min anaesthesia with unbuffered MS 222, neutralized MS 222 and benzocaine with and without physical stress. Blood samples were taken through cannulae inserted into the doral aorta. The Hct values and Hb concns increased with all the anaesthetics, which also caused swelling of erythrocytes. The initial values were restored within 4-12 h of recovery. Each anaesthetic elevated the blood lactate concn, but the initial level was regained within 12 hr. The blood glucose level decreased the most during anaesthesia with unbuffered MS 222, but the initial level was rapidly restored. Benzocaine caused the least hypoglycaemia during anaesthesia, but the subsequent hyperglycaemia, as in the fish anaesthetized with neutralized MS 222, lasted 24 hr. Neutralized MS 222 and benzocaine elevated the plasma K^+ concn more rapidly than unbuffered MS 222. The initial levels were regained in 4 days. All anaesthetics raised the Mg^{++} concn. The pO_2 in the dorsal aorta decreased during anaesthesia with unbuffered MS 222 by about 85 mmHg, while the pCO_2 increased by about 1.5 mmHg. Their initial levels were regained within 20 min. During anaesthesia the pH value decreased by 0.3 units and returned to the initial value within 2-4 hr of recovery. MS 222 seemed to be an asphyxiant.
Descriptors: anaesthesia, blood, biological stress, hypoxia, *Oncorhynchus mykiss*
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Soto C (1995) **Clove oil: A fish anaesthetic.** *Window newsletter. Mombasa.* 6(2):2-3
ISSN: 1024-4158
In experiments in Ambon, Indonesia, clove oil was used successfully to anaesthetise rabbit fish, *Siganus lineatus* in order to measure weight and length. Clove oil which has been used in Indonesia for centuries by humans as topical anaesthetic for toothaches, headaches and joint pains, is a dark brown liquid resulting from distillation of flowers, flower stalks and leaves of clove trees, *Eugenia aromatica*, and contains the compound eugenol. The stock solution of the anaesthetic (10g/l) was prepared by mixing 10ml of clove oil, 'Minyerk Cengkeh Merpati Putih' brand (composition 100% oleum chlorophyll) in one litre of boiled freshwater assuming the density of clove oil is approximately 1.0g/ml). The stock solution was shaken vigorously before mixing with seawater. The concentration of clove oil which fish lost consciousness within 1-1.5 minutes was 100mg/l (30ml stock solution in 3 litres).

Fish recovered consciousness quickly, within 3 minutes. Clove oil needs to be assessed as an anaesthetic for fish according to relevant criteria of efficacy, availability, ease of use, cost and side effects on fish, humans and the environment.
Descriptors: botanical resources, drugs, anaesthetics, anaesthesia, fish culture, aquaculture, *Siganus lineatus, Eugenia aromatica*, ISEW, Indonesia, clove oil, rabbit fish
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Soto CG, Burhanuddin (1995) **Clove oil as a fish anaesthetic for measuring length and weight of rabbitfish (*Siganus lineatus*).** *Aquaculture.* 136(1-2):149-152
NAL Call No. SH1A6
The successful use of clove oil as a fish anaesthetic is described. Juvenile rabbitfish, *Siganus lineatus*, from the same cohort ranging in size from 5 to 23 cm in total length were anaesthetized, and their length and weight were recorded on three separate occasions. Fish fed shortly afterward, and no mortality was observed. Clove oil appears to be highly effective as a fish anaesthetic with potentially few or no side effects. In Indonesia, its use is advantageous because it is locally produced and inexpensive.
Descriptors: anaesthetics, *Siganus lineatus*, aquaculture techniques, Indonesia, fish culture, clove oil
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Spotte S, Bubucis PM, Anderson G (1991) **Plasma cortisol response of seawater-adapted mummichogs (*Fundulus heteroclitus*) during deep MS-222 anesthesia.** *Zoo Biology* 10(1):75-79
NAL Call No. QL77.5.Z6
Anesthetics are used to reduce stress in fishes during handling and transfer. However, deep anesthesia of seawater-adapted mummichogs (*Fundulus heteroclitus*) results in a time-related increase in plasma cortisol, indicating a primary (neuroendocrine) stress response. Groups of seven fish were bled within 1 to 12 min of exposure to the anesthetic MS-222. Plasma cortisol rose more rapidly in fish removed from the MS-222 solution immediately after 1 min and held between wet paper towels than in fish that remained immersed. The difference between methods was significant ($P < 0.001$) with variation restricted to the later sampling periods. Differences were not significant in fish sampled immediately after 1 min.
Descriptors: serological studies, steroids, biological stress, anaesthetics, *Fundulus heteroclitus*, fish physiology, MS-222
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Stabell OB, Aanesen RT, Eilertsen HC (1999) **Toxic peculiarities of the marine alga *Phaeocystis pouchetii* detected by in vivo and in vitro bioassay methods.** *Aquatic Toxicology.* 44(4):279-288
NAL Call No. QH541.5.W3A6
The marine alga *Phaeocystis pouchetii* has recently been shown to display toxic properties towards fish (i.e. cod, *Gadus morhua*) larvae. The assumed toxic principle of this prymnesiophyte was extracted from *Phaeocystis* cultures by filtering and solid phase sorbent techniques. Toxicity testing was carried out by in vitro and in vivo bioassay methods based on blood haemolysis and injection into flies. The active material from the *Phaeocystis* cultures was found within a chemical fraction previously established for the separation of *Chrysochromulina sp.* and *Prymnesium sp.* toxins. The presence of active material was also found in filtered seawater collected during a *Phaeocystis* bloom, confirming that *Phaeocystis* releases the active material into the natural environment. Haemolytic activity was almost

absent in the material tested, demonstrating that the toxic principle in *Phaeocystis* is different from that described for other prymnesiophytes. By the fly bioassay method, on the other hand, a rapid response to injected material was obtained, resulting in a high proportion of apparently 'dead' flies registered within 1 h. The time scale of response in flies coincided with that previously reported for haemolytic toxins of *Prymnesium parvum*. However, an unexpected response was observed with the *Phaeocystis* material, since some of the flies that were assumed dead regained motility within a 4-h period of monitoring, the portion of awakened flies being inversely related to dose injected. Regained motility was also found with injected material from filtered natural seawater. Accordingly, the proposed toxins released by *Phaeocystis* appear to be compounds that hold anaesthetic properties, possibly expressing toxic effects when presented in surplus doses. These findings suggest that *Phaeocystis* may primarily be harmful to fish larvae following ingestion.
Descriptors: biological poisons, metabolites, anaesthetics, bioassays, seaweeds, fish larvae, algae, toxins, toxicity testing, hemolysis, algal blooms, bioassay, analytical methods, toxicity, marine organisms, larvae, ingestion, aquatic organisms, Pisces, *Phaeocystis pouchetti, Phaeocystis pouchetii, Gadus morhua*, Atlantic cod
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Stech L, Mims SD, Shelton WL, Linhart O (1998) **A modified method for removal of ovulated eggs from paddlefish.** *Aquaculture '98 Book of Abstracts*. p. 367
Paddlefish eggs when ovulated are discharged into the body cavity and exit through one of two ovarian funnels. Two methods are currently practiced in artificial propagation to remove the eggs from an ovulating fish: hand-stripping or caesarean section. Hand stripping is labor intensive often taking 8 to 10 hours to remove the eggs. Caesarean section is a quick method to remove the eggs in 10 to 15 minutes, but often the broodstock do not survive. Because of complications with both methods for egg removal, a new experimental procedure which in minimally invasive was developed for quick removal of eggs from an ovulated paddlefish. Ovulated female was anesthetized with 80 mg/l of MS-222 and placed ventral side up on a stretcher. A finger was inserted into the gonopore to enlarge the opening. A scalpel (no. 11 blade) was carefully inserted into the opening and a 1 to 2 cm incision was made through the oviduct (Mullerian duct) wall. After removal of the scalpel, the incision was probed with an inserted finger to insure that the opening permitted free copyright flow of eggs. The fish was inverted and pressure placed on the abdomen region by two personnel so the eggs flowed out the gonopore. Ten fish were ovulated using this method and time required to remove the eggs was 7 to 10 min/fish. Three of the fish were sacrificed to observe the effect from this method. Only a small quantity of eggs were retained in these fish. The other fish were stocked in ponds without any ill effects and will be used as potential broodstock in the future.
Descriptors: fish eggs, methodology, females, seed collection, ovulation, brood stocks, anaesthesia, aquaculture techniques, *Polyodon spathula*, USA, paddlefish
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Stehly GR, Gingerich WH (1999) **Evaluation of AQUI-S (efficacy and minimum toxic concentration) as a fish anaesthetic/sedative for public aquaculture in the United States.** *Aquaculture Research.* 30(5):365-372
NAL Call No. SH1 F8
A preliminary evaluation of efficacy and minimum toxic concentration of AQUI-S, a fish anaesthetic/sedative, was determined in two size classes of six species of fish important to US public aquaculture (bluegill, channel catfish, lake trout, rainbow trout, walleye and yellow perch). In addition, efficacy and minimum toxic concentration were determined in

juvenile-young adult (fish aged 1 year or older) rainbow trout acclimated to water at 7°C, 12°C and 17°C. Testing concentrations were based on determinations made with range-finding studies for both efficacy and minimum toxic concentration. Most of the tested juvenile-young adult fish species were induced in 3 min or less at a nominal AQUI-S concentration of 20 mg/L. In juvenile-young adult fish, the minimum toxic concentration was at least 2.5 times the selected efficacious concentration. Three out of five species of fry fingerlings (1.25-12.5 cm in length and < 1 year old) were induced in less than or equal to 4.1 min at a nominal concentration of 20 mg/L AQUI-S, with the other two species requiring nominal concentrations of 25 and 35 mg/L for similar times of induction. Recovery times were less than or equal to 7.3 min for all species in the two size classes. In fry-fingerlings, the minimum toxic concentration was at least 1.4 times the selected efficacious concentration. There appeared to be little relationship between size of fish and concentrations or times to induction, recovery times and minimum toxic concentration. The times required for induction and for recovery were increased in rainbow trout as the acclimation temperature was reduced.
Descriptors: anesthetics, sedatives, fish culture, aquaculture techniques, anaesthesia, public health, toxicology, toxicity tests, *Stizostedion vitreum vitreum, Perca flavescens, Oncorhynchus mykiss, Ictalurus punctatus, Salvelinus namaycush, Lepomis macrochirus*, USA, bluegill, rainbow trout, channel catfish, graceful catfish, lake trout, walleye, yellow perch
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Stone DS, Tostin N (1999) **Clove bud oil a big yawn for silver perch.** *Fisheries NSW. Sydney.* 2(4):19
ISSN: 1329-8267
Anaesthetics are used in aquaculture to minimize stress and damage to fish during harvesting, grading, transportation, spawning induction and handling. AQUI-S clove bud oil and benzocaine are among some of the anaesthetics currently being considered for use in the silver perch industry. At present, AQUI-S is the only anaesthetic registered with the National Registration Authority for use for food fish in Australia. The required concentration of each of several anaesthetics was evaluated to induce suitable levels of anaesthesia for the handling of silver perch (*Bidyanus bidyanus*) during harvesting or spawning induction. The study determined effective concentrations for three anaesthetics for harvesting and spawning induction for silver perch. All anaesthetics were reliable and easy to use, but clove bud oil was the most efficient and economical. For clove bud oil to be used as an anaesthetic for silver perch in the future, there is an urgent need for its registration with the National Registration Authority.
Descriptors: anaesthetics, fish handling, harvesting, anaesthesia, aquaculture techniques, fish culture, biological stress, food fish, *Bidyanus bidyanus*
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Strange RJ, Schreck CB (1978) **Anesthetic and handling stress on survival and cortisol concentration in yearling chinook salmon (*Oncorhynchus tshawytscha*).** *Journal of the Fisheries Research Board of Canada* 35(3):345-349
NAL Call No: 442.9 C16J
Brief anesthetization with 50 mg/L buffered MS-222 RegTM (ethyl m-aminobenzoate methanesulfonate) of yearling chinook salmon during mild handling caused no change in plasma cortisol concentrations compared with levels in non-anesthetized fish. Prolonged exposure (180 min) to a depressing dose of buffered MS-222 RegTM (25 mg/L) elevated

cortisol more than an immobilizing dose (50 mg/L), while 100 mg/L was lethal within 30 min. Fish anesthetized (50 mg/L MS-222 RegTM) during a severe 30-min handling stress had significantly lower mortality than controls to a second handling stress applied when the fish were no longer anesthetized. Anesthetization during the first stressor also prevented the cortisol stress response evident in the control fish. Anesthetic (with or without buffer) administered before initial capture was most effective at increasing survival during a second stressor, while anesthetic supplied after initial capture may have been slightly less effective. A 0.5% NaCl solution supplied after capture was less effective than any anesthetic treatment in increasing future survival, but was better than no treatment. Saline treatment did not attenuate the cortisol stress response. A rapid method of plasma sample preparation for competitive protein binding assay of cortisol was developed.
Descriptors: anaesthetics, fish handling, fatigue (biological), *Oncorhynchus tshawytscha*, survival, blood, bioassays, fishery management, fish culture
ASFA; Copyright © 2003, FAO

Suzuki A, Sekizawa Y (1979) **Residue analyses on 2-amino-4-phenylthiazole, a piscine anesthetic, in fishes. 4. GC/MS analysis on rainbow trout.** *Bulletin of the Japanese Society of Scientific Fisheries.* 45(2):167-171
NAL Call No. 414.9 J274
When a piscine anesthetic, 2-amino-4-phenylthiazole, was absorbed by rainbow trout, *Salmo gairdneri irideus*, from an anesthetic solution, a whole body concentration of 6.7 and 13 ppm was attained at 3 hours and 6 hours, respectively. After anesthetization for 6 hours, fish were transferred into 500 l of fresh water flowing at a rate 2 L per minute. Residues of the anesthetic in the fish were measured by gas chromatography-mass spectrometry (GC/MS). The total concentration of anesthetic in the fish declined at a rate comparable to that predicted by a computed theoretical biexponential curve obtained by a previously described mathematical procedure. The biological half-life of the anesthetic under these experimental conditions was approximately 40 minutes in the primary step and 22 hours in the secondary step. The whole body residues which remained at 24 and 48 hours were 0.1 ppm and 0.05 ppm, respectively.
Descriptors: anesthetics, excretion, body burden, biological half life, *Salmo gairdneri irideus*, 2-amino-4-phenylthiazole
ASFA; Copyright © 2003, FAO

Suzuki A, Shimura M, Kikuchi T, Sekizawa Y (1977) **Residue analyses on 2-amino-4-phenylthiazole, a piscine anesthetic, in fishes. 3. Metabolism in rainbow trout and carp.** *Bulletin of the Japanese Society of Scientific Fisheries.* 43(7):837-847
NAL Call No. 414.9 J274
The major biotransformation product of 2-amino-4-phenylthiazole in rainbow trout (*Salmo gairdneri irideus*) was isolated from water following exposure of fish to the anesthetic. The isolated crystalline metabolite was shown by means of ultraviolet, infrared and optical rotatory dispersion spectroscopy and gas chromatography to be identical to 2-amino-4-phenylthiazole-2-N- beta -mono-D-glucopyranosiduronic acid, the major biotransformation product previously found in medaka (killifish, *Oryzias latipes*). The major biotransformation product in carp (*Cyprinus carpio*) was also identified as 2-amino-4-phenylthiazole-2-N- beta -mono-D-glucopyranosiduronic acid by molecular sieve, thin layer and gas chromatography. Conversion of 2-amino-4-phenylthiazole to the N-glucuronyl conjugate was 8 and 12%, respectively, in rainbow trout and carp as shown by thin layer chromatography of extracts from fish treated with ^{3}H-labeled anesthetic. In addition, a minor metabolite of the anesthetic

in rainbow trout was isolated as a yellowish-white crystalline powder and identified as 2-acetamido-4-(4'-hydroxyphenyl)-thiazole by means of ultraviolet and infrared spectroscopy, NMR and mass spectrometry. Chromatography suggested that this same metabolite was also formed in carp but in concentrations too low for isolation and definitive identification.
Descriptors: anaesthetics, metabolism, *Salmo gairdneri, Cyprinus carpio*

Svobodova Z, Kalab P, Dusek L, Vykusova B, Kolarova J, Janouskova D (1999) **The effect of handling and transport on the concentration of glucose and cortisol in blood plasma of common carp.** *Acta Veterinaria (Brno).* 68:265-274
NAL Call No. SF604 B7
The aim of this contribution was to assess the degree of stress in common carp (*Cyprinus carpio*, L.) exposed to handling and transport. Cortisol and glucose concentrations in blood plasma were used as stress indicators. In some cases, concentration of ammonia in blood plasma and relative weight of spleen (SSI) were used as well. Within handling, an effect of time pause (0; 2 and 5 min) between catching the fish from water and blood sampling for stress indicators was checked. Another goal was to assess the effect of Menocain anaesthetic on the stress indicators. An open system of a 10-hour transport in a special long-distance live fish transport truck (Transport I a II), and a 2-hour transport in classic transporting tanks (Transport III) were compared. Temperature and oxygen concentration in water was measured during transport. After handling the fish prior to blood sampling (2 and 5 min pause), the cortisol concentration dropped significantly ($p< 0.001$) and the glucose concentration significantly ($p< 0.001$) increased compared to values in fish sampled immediately after catching. No effect of anaesthetics on cortisol concentration was proved. On the contrary, the glucose concentration fell ($p< 0.012$) in the anaesthetized fish. After a 10-hour transport in a special truck (Transport I), a significant ($p< 0.001$) decrease of the cortisol concentration was found, as well as a significant ($p< 0.001$) increase in the glucose concentration in blood plasma. In the course of 10-hour transport in a special truck (Transport II), the majority of carp was found dead. Suffocation and ammonia autointoxication due to loading the fish with full digestive tract were the most probable causes of the fish mortality. After a 2-hour transport in transporting tanks (Transport III) both the cortisol concentration and relative weight of spleen (SSI) dropped non-significantly while glucose concentration in blood plasma significantly ($p< 0.001$) increased. Results showed that both handling and transport are important stressors in the common carp. Preventive measures were proposed aimed at alleviating the negative effects of these stressors.
Descriptors: biological stress, anaesthetics, fish culture, transportation, glucose, blood, ammonia, spleen, *Cyprinus carpio*, cortisol, common carp, European carp, handling stress

Svobodova Z, Valentova V, Kouril J, Vykusova B, Hamackova J (1988) **The comparison of the effect of three anaesthetics on some hematological indicators and acidobasic balance in tench.** *Bulletin of the Fisheries Research Institute, Vodnany.* 24(1):10-17
NAL Call No. SH1 B77
An evaluation of the new Czechoslovak anaesthetic Menocain and a study of its effect on some hematological indicators and acidobasic balance in fish was carried out. The model fish in this comparative trial were female and male brood tench, Tinca tinca, at artificial spawning. In the fish anaesthetized by Menocain, no significant changes in the observed hematological indicators were recorded. On the basis of the carried out investigation with foreign preparations (Propoxate, Propanidide) it can be concluded that the new Czechoslovak

anaesthetic Menocain is a very suitable preparation for anaesthesia of carp fish from the aspect of safety.
Descriptors: anaesthesia, anaesthetics, aquaculture techniques, *Tinca tinca*, fish physiology, fish culture, Czechoslovakia, haematology, menocain, propoxate, propanidide

Svobodova Z, Valentova V, Vykusova B, Pecena M (1986) **Ichtyotoxicological evaluation of the new Czechoslovak anaesthetic for fish.** *Bulletin of the Fisheries Research Institute, Vodnany.* 23(1):3-7
NAL Call No. SH1 B77
The Czechoslovak anaesthetic for fish introduced under the title of Monetan has been evaluated. The tests were carried out in carp (*Cyprinus carpio*). The methodology was based on the data recommended by the authors of the new Czechoslovak anaesthetic to achieve anaesthesia in carp, that is the dose of 0.1 g per 1 L of water at the temperature of 18-24°C in time period of 10 min. LC_{50} value of Monetan for carp fry (K_1) in time period of 10 min and water temperature of 22°C was 0.81 g/L, minimum lethal concentration LC_5 in time period of 10 min and water temperature of 22°C was 0.49 g/L. T_{50} value of Monetan (i. e. the time in the course of which 50% die using the recommended dose of 0.1 g/L and water temperature of 22°C) was 101 min, t_5 value (i. e. the time in the course of which 5% die using the recommended dose of 0.1 g/L and water temperature of 22°C) was 83 min.
Descriptors: anesthesia, hematology, lethal limits, *Cyprinus carpio*, anesthetics, toxicity tolerance, test organisms, Czechoslovakia, Monetan

Svobodova Z, Vykusova B, Kouril J (1992) **Menocain -- Czechoslovak anaesthetics for fish.** *Proceedings of The Scientific Conference on Fish Reproduction '92 (Vodnany, Czechoslovakia, 2-4 March, 1992)., Res. Inst. of Fish Culture and Hydrobiol., Vodnany (Czech Rep.).* pp. 152-153
Menocain is a specific fish anaesthetics which does not provoke anaesthesia in homoiothermic animals. There was confirmed on the base of toxicological, hematological, and histopathological investigations that this anaesthetics is safe for fish. Its suitability for fish anaesthesia was proved by several years of its successful application in fishery practice.
Descriptors: fish culture, aquaculture techniques, anaesthetics, haematology, histopathology, toxicology, Menocain

Sylvester JR, Holland LE (1982) **Influence of temperature, water hardness and stocking density on MS-222 response in three species of fish.** *Progressive Fish-Culturist.* 44(3):138-141
NAL Call No. 157.5 P94
Three responses of rainbow trout (*Salmo gairdneri*), common carp (*Cyprinus carpio*), and fathead minnows (*Pimephales promelas*) to the anesthetic MS-222 (tricaine methanesulfonate) were identified: induction times to total loss of equilibrium decreased with increasing temperatures; resistance to MS-222 increased with increasing water hardness; and resistance to the drug increased when the densitv of test fish was increased from 0.3 to 0.9 g/L in rainbow trout, 0.4 to 1.2 g/L in carp, and 0.3 to 0.8 g/L in fathead minnows. These results, and others from the literature, suggest that temperature, water hardness, and density of test fish, as well as pH and dissolved oxygen, may have significant interacting effects on the anesthetic properties of MS-222.
Descriptors: temperature effects, water hardness, stocking density, anesthetics, *Salmo*

gairdneri, Cyprinus carpio, Pimephales promelas, response analysis, environmental effects
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Takashima Y, Wan Z, Kasai H, Asakawa O (1983) **Sustained anesthesia with 2-phenoxyethanol in yearling rainbow trout.** *Journal. Tokyo University of Fisheries.. Tokyo Suisandai Kempo.* 69(2):93-96
ISSN: 0040-9014
The possibility of using 2-phenoxyethanol for long-term anesthesia in yearling rainbow trout, *Salmo gairdneri*, was examined. The 24 hour TLm was 320 ppm for trout yearling (100-200 g in body weight, at 12°C) and none of the fish immersed below 200 ppm died until 24 hours. All of the fish anesthetized below 200 ppm showed normal appearance and behavior after transferring to clean running water. Therefore, it was thought to be possible to anesthetize with 2-phenoxyethanol for at least 24 hours. On the other hand, serum cortisol levels analyzed by radioimmunoassay increased rapidly after immersion into 150 ppm of 2-phenoxyethanol for 1 hour. Moreover, prominent elevation of serum cortisol level was also recognized following mechanical agitation of rearing water in spite of anesthetic condition. It may be concluded that anesthesia with 2-phenoxyethanol does not reduce the stress during transport.
Descriptors: fish physiology, anesthesia, stress, fish handling, biological stress, *Salmo gairdneri*, phenoxyethanol
ASFA; Copyright © 2003, FAO

Tamaru CS, Carlstrom-Trick C, FitzGerald WJ Jr (1996) **Clove oil, minyak cengkeh, a natural fish anesthetic.** *Proceedings of the Pacon Conference on Sustainable Aquaculture '95*.pp. 365-371
NAL Call No. TC1505 P33 1995
Indonesian clove oil, minyak cengkeh, was found to be an effective anesthesia for use in hatchery practices with the rabbit fish *Siganus argenteus*. At a concentration of 25 ppm fish could be physically handled for length and weight determinations and for the performance of gonadal biopsy. Time to loss of equilibrium was less than three minutes at the lowest concentration tested and time to recovery was less than five minutes at all concentrations tested. No adverse effects or mortalities were observed during the two month study period. Clove oil was also found to be more effective than 2-phenoxyethanol under the same conditions and at the same dosages. The use of minyak cengkeh is a perfect example of how we in the developed countries tend to overlook the available resources in recipient countries of our technology transfer activities. Technology transfer, or technical assistance, most often involves the transfer of technology developed in a developed country to an undeveloped or a developing country by so-called experts in the developed country that developed the technology. Because the technology transferred is too often unfamiliar to the recipient, the materials needed to sustain the technology too often unavailable, and chronically the resources in the developing country are scrapped for the modern and more sophisticated ways of the "Western World", we may have taught someone how to cook a gourmet dinner but he still won't be able to feed the family.
Descriptors: fish culture, hatcheries, aquaculture techniques, anaesthetics, *Siganus argenteus*
ASFA; Copyright © 2003, FAO

Taylor PB (1988) **Effects of anaesthetic MS 222 on the orientation of juvenile salmon, *Oncorhynchus tshawytscha* Walbaum.** *Journal of Fish Biology.* 32(2):161-168
NAL Call No. QL614 J68

Juvenile salmon, *Oncorhynchus tshawytscha*, were trained to orientate in a direction (270 degree) and then anaesthetized with ethyl m-aminobenzoate methane sulphonate (MS 222) in a test to determine whether anaesthesia affected the learnt orientation. Before anaesthesia the control group of twelve fish showed a mean unimodal orientation of 264°and a bimodal axis of orientation of 258 °/78° with a confidence limit (second order analysis) of between 218 degree and 285°. After administration of MS 222 nine out of ten fish showed marked changes in orientation and random behaviour, persisting in two fish for more than 14 days. Eight weeks after completion of the anaesthesia trial the fish were retested. Each fish, except one, showed a mean bimodal axis of orientation that fell within the confidence limit of the control. The results support the view that the reference orientation in this study (270°) was a learnt, not an innate directional preference. The effects anaesthesia may have on salmon behaviour during migration are discussed.
Descriptors: anaesthetics, orientation behaviour, learning behaviour, *Oncorhynchus tshawytscha*, migrations
ASFA; Copyright © 2003, FAO

Tsuda Heizo (1987) **Experiments on the method of anesthetization of juvenile bluefin tuna.** *Bulletin of the Fisheries Research Institute, Mie, Japan.* 2:9-18
The juvenile bluefin tuna are weak in the skin, which is an obstacle to the research procedure such as weighing, measuring, transferring and so on. To safeguard the tuna against abrasion, the useful anesthesia has been studied over the period of 1983 to 1985. The tuna cultured in the tank, 210 g to 1970 g by weight, were used. Ulethane, MS-222 (m-aminobenzoic acid ethylester metanesulfonate), and eugenol (4-allyl-2-methoxyphenol) were examined as to the anesthetic effects to the tuna. In addition to this, when using cooled sea water and cooled sea water added ulethane, the effect was examined. The tuna were caught one by one with rod from the tank and transferred to the anesthetic solution prepared. After anesthetized, the tuna were weighed and moved back to the sea water, and then recovered from anesthesia. <<Abstract is partly entered herein due to the whole abstract is too long.>>
Descriptors: tuna fisheries, fish larvae, fish culture, anaesthesia, anaesthetics, *Thunnus thynnus*, bluefin tuna, urethane, MS-222, Eugenol
ASFA; Copyright © 2003, FAO

Tytler P, Hawkins AD (1981) **Vivisection, anaesthetics and minor surgery.** *Aquarium Systems.*, pp. 247-278.
NAL Call No. SF457 A67
The following topics are considered: vivisection, immobilization and anaesthesia, humane killing of fish, surgery and surgical equipment, marking fish, electrocardiography, blood sampling and physiological saline solution.
Descriptors: fish handling, experimental research, anesthesia
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Umbreit N (1980) **Chemical Restraint of Reptiles, Amphibians, Fish, Birds, Small Mammals and Selected Marine Mammals in North America: An Annotated Bibliography (Technical note)**. Bureau of Land Management, Denver, CO. *Report Number: TN-340*,181 p.
Order this product from NTIS by: phone at 1-800-553-NTIS (U.S. customers); (703)605-6000 (other countries); fax at (703)321-8547; and email at orders@ntis.fedworld.gov.
This annotated bibliography provides a list of available literature sources on anesthetizing or immobilizing small animals. Although the original intent of this bibliography was to deal

strictly with small wild animals, it was expanded to include listing on many laboratory animals such as rabbits, rats, and mice. Extensive testing and research on these animals has led to better methods in handling, anesthetizing and immobilizing. For these reasons, it is hoped that the procedures and methods obtained in the laboratory may apply to the welfare, well-being and management of wild animal populations. Information has also been included on repellants, as they too are a form of chemical restraint.
Descriptors: mammals, wildlife, laboratory animals, constraining, bibliographies, amphibia, fish, birds, reptiles, tranquilizer drugs, anesthetics, repellants, dosage

Veenstra RS, Balon EK, Flegler-Balon C (1987) **Propanidid, a useful anaesthetic for studying blood circulation in early development of fish.** *Canadian Journal of Zoology.* 65(5):1286-1289
NAL Call No. 470 C16D
The effectiveness of propanidid was tested by comparing it with cocaine hydrochloride, urethane, and traicaine methanesulfonate, anaesthetics already established for studies of early ontogeny in fishes. Free embryos of the brook charr, *Salvelinus fontinalis*, and 7-day-old amargosa pupfish, *Cyprinodon nevadensis amargosae*, were anaesthetized with dilute solutions of these drugs. The times taken until the blood elements ceased moving through the capillary loops of the developing caudal fin were compared. Propanidid was found to be superior to the other drugs tested in maintaining the longest duration of unaltered blood flow.
Descriptors: anaesthetics, methodology, comparative studies, *Salvelinus fontinalis*, *Cyprinodon nevadensis amargosae*, blood circulation, propanidid
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Wagner E, Arndt R, Hilton B (2002) **Physiological stress responses, egg survival and sperm motility for rainbow trout broodstock anesthetized with clove oil, tricaine methanesulfonate or carbon dioxide.** *Aquaculture.* 211(1-4):353-366
NAL Call No. SH1A6
Egg survival, sperm motility and physiological stress responses (plasma cortisol, glucose and chloride) of rainbow trout (*Oncorhynchus mykiss*) broodstock were compared among three anesthetics: tricaine methanesulfonate (MS-222), clove oil in the form of AQUI-S (a proprietary mix of 50% isoeugenol and other ingredients) and carbon dioxide gas. Concentrations of 60 mg/l tricaine, 20 mg/l isoeugenol (40 mg/l AQUI-S) and 220-275 mg/l carbon dioxide were based on preliminary tests and chosen to standardize induction time among anesthetics. Plasma glucose, chloride and cortisol concentrations indicated that none of the anesthetics used after crowding and netting completely eliminated the stress response. The return to prestress cortisol levels differed among the three anesthetics. Fish anesthetized with AQUI-S had significantly lower cortisol concentrations at 1 or 7 h postimmersion than the other anesthetics and controls, but were elevated at 24 h. Plasma cortisol in tricaine- and CO_2-treated fish returned to prestress levels within 7 and 24 h, respectively, whereas cortisol levels in control fish remained elevated at 24 h. Sperm motility and duration of motility were assessed for a practical range of concentrations: tricaine, 15-100 mg/l; AQUI-S, 10-100 mg/l; CO_2, 50-173 mg/l. The percentage of motile sperm was unaffected by anesthetic treatment, averages ranging from 68% to 87%. However, duration of motility decreased as anesthetic concentration increased, averages ranging from 55 to 36 s for tricaine and from 56 to 37 s for AQUI-S. Duration of sperm motility was low (31-43 s) for all levels of CO_2 tested. Fish recovery time was significantly longer for fish anesthetized by AQUI-S (370 s) than the either CO_2 or tricaine (192 and 199 s, respectively). Gender had no effect on recovery time. Egg survival to the eyed stage and to hatch was not significantly different among anesthetic

treatments and controls. No delayed mortality was observed for any of the fish handled and bled for the test. Results indicated that tricaine, AQUI-S and CO2 were all suitable for broodfish anesthesia, but the longer recovery time and lower cost for AQUI-S may make it more useful than the alternatives. None of the anesthetics wholly suppressed the stress responses during a typical spawning process, but did help reduce the duration of the stress responses and eased handling without compromising egg viability.
Descriptors: brood stocks, fish eggs, sperm, aquaculture techniques, induced breeding, anaesthesia, handling, carbon dioxide, biological stress, locomotion, survival, biological fertilization, *Oncorhynchus mykiss*, cortisol, clove oil, rainbow trout, tricaine methanesulfonate
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Walsh CT, Pease BC (2002) **The use of clove oil as an anaesthetic for the longfinned eel, *Anguilla reinhardtii* (Steindachner).** *Aquaculture Research.* 33(8):627-635
NAL Call No. SH1 F8
To handle large river eels during procedures such as measuring and tagging for field and aquaculture studies, they must be anaesthetized. During our initial biological studies of *Anguilla reinhardtii* (Steindachner) it was found that the anaesthetic benzocaine was relatively expensive and elicited a variable response, even when used at relatively high concentrations. Human health risks are also a concern when using benzocaine, as some of the eels may later be sold for human consumption. Therefore, experiments were done to evaluate the use of clove oil (a safe, naturally occurring product) for anaesthesia of this species at a range of temperatures (17 and 25°C) and salinities (0-32 g/L. It was found that clove oil provided a suitable anaesthetic response through this wide range of temperatures and salinities. Response times were found to be relatively variable for both benzocaine and clove oil. This variability may be related to stress, environmental factors, or the condition of the fish. Clove oil is recommended for anaesthesia of anguillid eels because it is effective, relatively inexpensive, and poses little risk to human health.
Descriptors: costs, aquaculture techniques, anaesthetics, Anguillidae, *Anguilla reinhardtii, Syzygium aromaticum*, clove oil, freshwater eels
ASFA; Copyright © 2003, FAO

Waterstrat PR (1999) **Induction and Recovery from Anesthesia in Channel Catfish *Ictalurus punctatus* Fingerlings Exposed to Clove Oil.** *Journal of the World Aquaculture Society.* 30(2):250-255
NAL Call No. SH138 W62
The use of clove oil as an anesthetic for channel catfish *Ictalurus punctatus* fingerlings was examined. At 100 mg/L, clove oil induced anesthesia within 1 min following exposure. Fish recovered from a 10-min period of anesthesia in 100 mg/L clove oil within 4 min following removal from the anesthetic solution. At clove oil concentrations of 150 mg/L or greater, recovery times were prolonged, requiring longer than 10 min for recovery. At 300 mg/L, mortality was observed with half of the catfish fingerlings failing to recover from the 10-min exposure. Fish could be safely maintained in 100 mg/L clove oil for periods of up to 15 min; exposure for longer than 15 min produced both prolonged recovery times and mortality. At a concentration of 100 mg/L clove oil produced responses similar to those of the commonly used fish anesthetic MS-222. The low cost of clove oil relative to MS-222 and the extensive testing and use of clove oil in dentistry and as a food ingredient make clove oil an attractive candidate as a fish anesthetic.
Descriptors: fish culture, anaesthesia, aquaculture techniques, fingerlings, mortality, *Ictalurus punctatus*, channel catfish
ASFA; Copyright © 2003, FAO

Weyl O, Kaiser H, Hecht T (1996) **'Breath holding' in tilapia *Oreochromis mossambicus*.** *South African Journal of Science.* 92(3):152-154
NAL Call No. 515 SO84
During experiments on the use of anaesthetics in fishes, it was observed that *Oreochromis mossambicus* was able to cease opercular movement for up to 15 minutes. It could be shown that this was not an immediate anaesthetic effect. The cessation of ventilation corresponded to a reduction of oxygen consumption. The pathway of detection of foreign substances appears to be olfactory. We discuss possible reasons for this specific, previously unobserved behaviour, as well as its consequences for culturing this and other fish species.
Descriptors: anesthetics, fish culture, oxygen consumption, olfaction, gills, respiration, *Oreochromis mossambicus*
ASFA; Copyright © 2003, FAO

Weyl O, Kaiser H, Hecht T (1996) **On the efficacy and mode of action of 2-phenoxyethanol as an anaesthetic for goldfish, *Carassius auratus* (L.), at different temperatures and concentrations.** *Aquaculture Research.* 27(10):757-764
NAL Call No. SH1 F8
The objective of this study was to assess the efficacy and mode of action of 2-phenoxyethanol as an anaesthetic for two size classes of goldfish, *Carassius auratus* (L.), at three different temperatures. Goldfish (2.15 ± 0.05 g, and 9.19 ± 0.17 g) were exposed to 0.3, 0.4 and 0.5 ml 2-phenoxyethanol/L at 20, 25, and 30°C. Time needed to induce anaesthesia was dependent on concentration and water temperature. At temperatures at and below 25°C, 0.4 ml/L was needed to induce total loss of equilibrium within less than 15 min. Above 25°C, 0.5 ml/L was required to induce anaesthesia. Recovery rate was independent of the length of anaesthesia, which indicates that the anaesthetic is taken up and lost via a concentration gradient at the gill membrane and skin/solution interface. Fish recovered within less than 10 min after they had been taken out of the anaesthetic solution. In a second experiment, goldfish responded to a repeated exposure to 2-phenoxyethanol daily over a period of 14 days with increased tolerance, which indicates a habituation response to the anaesthetic. The use of 2-phenoxyethanol as an anaesthetic both for short-term anaesthesia and for anaesthesia under transport conditions is discussed.
Descriptors: anaesthetics, bioassays, fish culture, *Carassius auratus*, 2-phenoxyethanol
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Yamamitsu S, Itazawa Y (1988) **Effects of an anesthetic 2-phenoxyethanol on the heart rate, ECG and respiration in carp.** *Nippon Suisan Gakkaishi/Bulletin of the Japanese Society of Scientific Fisheries.* 54(10):1737-1746
NAL Call No. 414.9 J274
Heart rate, ECG and respiratory parameters were measured with carp (*Cyprinus carpio*) of about 600 g during various stages of anesthesia induced with 400-800 ppm solution of 2-Phenoxyethanol. In 400 ppm solution, deep sedation, tachycardia, shortening of QT sub(1), increase in ventilation frequency and slight decrease in oxygen consumption were observed. In 600 ppm solution, disappearance of tachycardia, extension of time elements of ECG, and decrease in all respiratory parameters were observed. In anesthesia induced with 400-600 ppm solution, fish recovered from anesthesia by irrigation with fresh water containing no anesthetic. In 800 ppm solution, progressive bradycardia, remarkable extension of time elements of ECG and decrease in voltage elements of ECG, and drops to almost zero in respiratory parameters owing to cessation of ventilation movement were observed and fish did not recover from anesthesia by irrigation with fresh water.
Descriptors: fish physiology, bioassays, *Cyprinus carpio*, anaesthetics, 2-phenoxyethanol
ASFA; Copyright © 2003, FAO

Yokoyama Y, Yoshikawa H, Ueno S, Mitsuda H (1989) **Application of CO_2-anesthesia combined with low temperature for long-term anesthesia in carp.** *Nippon Suisan Gakkaishi/Bulletin of the Japanese Society of Scientific Fisheries.* 55(7):1203-1209
NAL Call No. 414.9 J274
The efficacy of CO_2 anesthesia combined with low temperature in long-term anesthesia supposing the transportation of live fish was evaluated using adult carp *Cyprinus carpio* acclimated at 23°C. All the carp became anesthetized in about 10 min with a 30-min cold treatment at 4°C and were safely maintained in an anesthetic state for the following 9.5 h with the cold-CO_2 treatment. The optimum anesthetic condition for adult carp was pCO_2 at 80 mmHg at 14°C.
Descriptors: carbon dioxide, water temperature, transportation, live storage, *Cyprinus carpio*, anaesthesia
ASFA; Copyright © 2003, FAO

Yoshikawa H, Ueno S, Mitsuda H (1989) **Short- and long-term cold-anesthesia in carp.** *Nippon Suisan Gakkaishi/ Bulletin of the Japanese Society of Scientific Fisheries.* 55(3):491-498
NAL Call No. 414.9 J274
The efficacy of cold anesthesia in the transportation of live fish was evaluated using carp (*Cyprinus carpio*) acclimated at 23°C. The carp could be safely maintained in an anesthetic state for 5 h in water kept at 4°C and in the anesthetized or sedated state for 24 h at 8-14°C. Some anesthetized carp showed signs of convulsion when they received external stimuli, and bled mainly from gills. Hemorrhage became distinct with the decrease in temperature and the duration of the cold treatment. The sedated carp showed no such excitement and bleeding. The sedated state is considered to be adequate for transportation, regardless of the anesthetic time; 14°C seems to be the optimal temperature when the carp are acclimated to 23°C.
Descriptors: temperature effects, *Cyprinus carpio*, transportation, anaesthesia, cold-anesthesia
ASFA; Copyright © 2003, FAO

Zinkovsky OG, Potrokhov AS, Evtushenko NYu (1995) **The use of tranquillizers and antidepressants in experimental studies of fish.** *Gidrobiologicheskij Zhurnal (Kiev) Hydrobiology Journal.* 31(3):85-94
NAL Call No. QH90 A1G5
A series of drugs (phenazepamum, hydazepamum, phenobarbital, aminazinum) were tested on common carp (*Cyprinus carpio*) and silver carp (*Hypophthalmichthys molitrix*) for the applicability in fish-breeding as anaesthetic agents and antidepressants. Tentative pharmacological assessment is given. Methods and techniques allowing employment of the drugs in fish-breeding and related activities are discussed.
Descriptors: biological stress, fish physiology, anaesthetics, *Cyprinus carpio*, *Hypophthalmichthys molitrix*, Ukraine
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Web Resources:

2000 Report of the AVMA Panel on Euthanasia
http://www.avma.org/resources/euthanasia.pdf

Anadromous Salmonid Passage Facility Guidelines and Criteria
http://www.nwr.noaa.gov/1hydrop/hydroweb/docs/release_draft.pdf

A draft document which prohibits the use of carbon dioxide anesthesia.

Canadian Council on Animal Care (CCAC)
http://www.ccac.ca

The Canadian Council on Animal Care (CCAC) has posted its second draft of CCAC guidelines on: the care and use of fish in research, testing, and teaching. The draft guidelines and future final guidelines are available at the CCAC webpage (http://www.ccac.ca and http://www.ccac.ca/english/new/newframe.htm).

CVM Guide 1240.4200
Low Regulatory Priority Aquaculture Drugs
http://www.fda.gov/cvm/index/aquaculture/LRPDrugs.pdf

CVM Guide 1240.4260
Classification of Aquaculture Species as Food or Nonfood
http://www.fda.gov/cvm/index/policy_proced/4260.pdf

Drugs Approved for Use in Aquaculture
http://www.fda.gov/cvm/index/aquaculture/appendixa6.htm

Endangered Species Act Section 7 Consultation Supplemental Biological Opinion
http://www.nwr.noaa.gov/1hydrop/hydroweb/docs/Final/chap11.pdf.

Biological opinion which mentions anesthetics in fish and the excessive thrashing caused by CO_2

FDA-CVM guidance document 150
Guidance for Industry, Status of Clove Oil and Eugenol for Anesthesia of Fish
http://www.fda.gov/cvm/index/updates/gl150.htm

FishDoc – The Home of Fish Health
http://www.fishdoc.co.uk/index.htm

Fish Anaesthetics:
Why use aneasethetics in treating fish disease?
http://www.fishdoc.co.uk/treatments/anaesthetics01.htm
Types of anaesthetics
http://www.fishdoc.co.uk/treatments/anaesthetics02.htm
How to use anaesthetics
http://www.fishdoc.co.uk/treatments/anaesthetics03.htm
For use in euthanasia
www.fishdoc.co.uk/treatments/euthanasia.htm

Food Animal Residue Avoidance Data bank (FARAD)
www.farad.org

MS222 (TRICAINE METHANE SULPHONATE)
http://www.alpharmaanimalhealth.co.uk/VPDF/MS%20222.pdf

> **...** transport Anaesthesia vaccination, minor surgery Deep Anaesthesia major surgery, euthanasia For further ... Anaesthesia and Restraint. In: Textbook of fish medicine ...

Recommendations for euthanasia of experimental animals: Part 1
http://www.ifp.kvl.dk/education/animal/Tekster/Euthanasia%201.pdf

Ross, L.G. & Ross, B. 1999. **Anaesthetic and Sedative techniques for Aquatic Animals. Second edition.** Fishing News Books. Blackwell Science. Oxford. pp176. ISBN 0-632 05252X
http://www.aquaculture.stir.ac.uk/GISAP/Pubs/A&S.htm

Ross, L.G. & Ross, B. 1984. **Anaesthetic and Sedative techniques for fish. Institute of Aquaculture Handbook**. University of Stirling. pp45. ISBN 0-901636-52-5.
http://www.aquaculture.stir.ac.uk/GISAP/Pubs/A&S.htm

Species Specific Anaesthesia
Woods Hole Oceanographic Institute
Bibliographic listing of resources
http://www.mblwhoilibrary.org/animal/seven.html

White Paper Summary Of Research Related To Transportation Of Juvenile Anadromous Salmonids Around Snake And Columbia River Dams
http://www.nwfsc.noaa.gov/publications/whitepapers/trans4-25-00.pdf

A letter (with several pieces of follow-up correspondence) from FDA to USFWS regarding the use of non-approved drugs on threatened and endangered species is freely available, but you must request these from **Dr. Dave Erdahl (406-587-9265 x125, dave_erdahl@fws.gov) of the USFWS** who has the current INAD for the use of isoeugenol.

3.1.3. AWARENESS, COGNITIVE ABILITY, & FEAR

Bisazza A, De Santi A, Vallortigara G (1999). **Laterality and cooperation: mosquitofish move closer to a predator when the companion is on their left side.** *Animal Behaviour.* 57:1145-1149
NAL Call No. 410 B77
Descriptors: mosquitofish, laterality, awareness, behavior, *Gambusia holbroki*

Bisazza A, Pignatti R, Vallortigara G (1997) **Detour tests reveal task- and stimulus-specific behavioural lateralization in mosquitofish (*Gambusia holbrooki*).** *Behavioural Brain Research.* 89:237-242
Descriptors: mosquitofish, laterality, awareness, behavior, *Gambusia holbroki*

Bisazza A, Rogers LJ, Vallortigara G (1998) **Origins of cerebral asymmetry: a review of evidence of behavioural and brain lateralization in fishes, reptiles and amphibians.** *Neuroscience and Biobehavioural Reviews.* 22:411-426
NAL Call No. QL750 B5
Descriptors: brain lateralization, awareness, behavior, fishes

Bunge M, Ardila R (1987) ***Philosophy of Psychology.*** Springer-Verlag, New York
Descriptors: brain lateralization, awareness, behavior, fishes

Canfield JG, Rose GJ (1993) **Activation of Mauthner neurons during prey capture.** *Journal of Comparative Physiology A.* 172:611-618
NAL Call No. 444.8 Z3
Descriptors: Mauthner neurons, prey capture, awareness, behavior, fishes

Cantalupo C, Bisazza A, Vallortigara G (1995) **Lateralization of predator-evasion response in a teleost fish (*Girardinus falcatus*).** *Neuropsychologia.* 33:1637-1646
Descriptors: mosquitofish, laterality, awareness, behavior, teleost, *Girardinus falcatus*

Cornish IME, Moon TW (1986) **The glucose and lactate kinetics of American eels, *Anguilla rostrata* (LeSueur), under MS 222 anaesthesia.** *Journal of Fish Biology.* 28(1):1-8
NAL Call No. QL614 J68
Glucose and lactate kinetics were examined in fed and food-deprived American eels, *Anguilla rostrata*, under MS 222 anaesthesia (AE). These values are compared to free-swimming, non-anaesthetized animals (FSE) reported previously (Cornish & Moon, 1985). The AE group demonstrated a steady but minor hyperglycemia during the 5-h experiment and significant decreases in both glucose turnover and metabolic clearance rates compared to the FSE groups. Food-deprivation further depressed these kinetic parameters. Blood lactate continuously increased during the experiment, reaching values 300 times (fed) and 100 times (food-deprived) higher than the similar FSE groups. Rates of lactate appearance in and disappearance from the blood generally increased with anaesthesia. This study supports the view of Soivio et al. (1977) that MS 222 acts as an asphyxiant.
Descriptors: fish physiology, kinetics, glucose, anesthesia, animal metabolism, *Anguilla rostrata*, lactate, hyperglycemia

Csanyi V (1986) **Ethological analysis of predator avoidance by the paradise fish (*Macropodus opercularis* L.): II. Key stimuli in avoidance learning.** *Animal Learning & Behavior.* 14:101-109
NAL Call No. QL785 A725
Descriptors: ethology, awareness, behavior, learning, paradise fish, *Macropodus opercularis*

Csányi V (1993) **How genetics and learning make a fish an individual: a case study on the paradise fish.** In: *Perspectives in Ethology. Volume 10; Behavior and Evolution* (ed. by P.P.G. Bateson, P.H. Klopher & N.S. Thompson), pp. 1-51. Plenum Press, New York
NAL Call No. QL751 P4
Descriptors: ethology, genetics, awareness, behavior, learning, paradise fish, *Macropodus opercularis*

Csányi V, Csizmadia G, Miklosi A (1989) **Long-term memory and recognition of another species in the paradise fish.** *Animal Behaviour.* 37:908-911
NAL Call No. 410 B77
Descriptors: ethology, awareness, behavior, learning, memory, recognition, paradise fish, *Macropodus opercularis*

Csányi V, Dóka A (1993) **Learning interactions between prey and predator fish**. *Marine and Freshwater Behaviour and Physiology.* 23:63-78
Descriptors: ethology, awareness, behavior, learning, predator-prey relationship

Csànyi V, Lovàsz F (1987) **Key stimuli and the recognition of the physical environment by the paradise fish *Macropodus opercularis*.** *Animal Learning & Behavior.* 15:379-381
NAL Call No. QL785 A725
Descriptors: ethology, awareness, behavior, environment, recognition, paradise fish, *Macropodus opercularis*

Davis RE, Klinger PD (1994) **NMDA receptor antagonist MK-801 blocks learning of conditioned stimulus-unconditioned stimulus contiguity but no fear of conditioned stimulus in goldfish (*Carassius auratus* L.).** *Behavioral Neuroscience.* 108:935-940
NAL Call No. QP351 B45
Descriptors: ethology, awareness, behavior, goldfish, *Carassius auratus*, MK-801

Dugatkin LA, Wilson DS (1994) **Choice experiments and cognition: A reply to Lamprecht and Hofer.** *Animal Behaviour.* 47(6):1459-1461
NAL Call No. 410 B77
Lamprecht & Hofer (1994) raise three criticisms of our study of bluegill cognitive abilities (Dugatkin & Wilson 1992), which are outlined as follows.
Descriptors: *Lepomis macrochirus*, cognitive ability, cooperativity, freshwater fish, intraspecific relationships, learning behavior, cooperation
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Eaton RC, Lavender WA, Wieland CM (1981) **Identification of Mauthner-initiated response in goldfish: evidence from simultaneous cinematography and electrophysiology**. *Journal of Comparative Physiology* 144:521-531
NAL Call No. 444.8 Z3
Descriptors: ethology, awareness, behavior, goldfish, *Carassius auratus*

Eaton RC, Lavender WA, Wieland CM (1982) **Alternative neural pathways initiate fast-start responses following lesions of the Mauthner neuron in goldfish**. *Journal of Comparative Physiology* 145:485-496
NAL Call No. 444.8 Z3
Descriptors: ethology, awareness, behavior, goldfish, neurobiology, Mauthner neuron

Eaton RC, Nissanov J (1985) **A review of Mauthner-initiated escape behaviour and its possible role in hatching in the immature zebrafish, *Brachydanio rerio***. *Environmental Biology of Fishes.* 12:265-279
NAL Call No. QL614 E56
Descriptors: ethology, awareness, behavior, zebrafish, *Danio rerio, Brachydanio rerio*, neurobiology, Mauthner neuron

Facchin L, Bisazza A, Vallortigara G (1999) **What causes lateralization of detour behavior in fish? evidence for asymmetries in eye use.** *Behavioural Brain Research.* 103:229-234
Descriptors: ethology, behavior, lateralization, eye

Gerlai R, Csanyi V (1994) **Artificial bidirectional selection for a species-specific behavioural element, staccato movement, in paradise fish, Macropodus opercularis.** *Animal Behaviour.* 48(6):1293-1300
NAL Call No. 410 B77
The possibility that phenotypical correlations between staccato movement (an element of fear response in novel situations) and several other behavioural elements of paradise fish have a genetic component has not been investigated previously. The difficulty in determining the genetic underpinnings of staccato movement has been due to violation of the criteria of additive-dominance genetic models of applied-classical and diallel-cross analyses. Furthermore, the limited number of available inbred strains in these cross systems could decrease the number of variable loci and thus one's ability to detect possible genetic effects including genetic covariation. To circumvent these problems, bidirectional selection using a genetically variable outbred population was carried out by recording the behaviour elements in a novel situation, and based on staccato performance, breeding two high-performer and two low-performer lines and a non-selected control population. The response to selection was immediate and strong in the first selected generation in both directions without significant change in the succeeding generations, suggesting a major gene effect in the inheritance of staccato movement. Phenotypical correlations were confirmed between staccato and several other elements. However, correlated response to selection was observed only in one behavioural element (swim), suggesting a genetic component in the swim-staccato phenotypical correlation due either to pleiotropic gene effects or to genetic linkage. The other significant phenotypical correlations between staccato and the behavioural elements, which were non-genetic in origin, also represent a stable correlation structure, which is interpreted to be the result of higher neural mechanisms.
Descriptors: fear, *Macropodus opercularis*, environmental effects, behavioral genetics, outbreeding, flight behaviour, genetics, marine fish, ethology, staccato movement, outbreeding

Hall D, Suboski D (1995) **Visual and olfactory stimuli in learned release of alarm reactions by zebra danio fish (*Brachydanio rerio*).** *Neurobiology of Learning and Memory.* 63:229-240
NAL Call No. QH301 C63

Descriptors: ethology, awareness, behavior, zebrafish, *Danio rerio, Brachydanio rerio*, neurobiology, visual stimuli, olfactory stimuli

Harper DG, Blake RW (1990) **Fast-start performance of rainbow trout *Salmo gairdneri* and Northern pike *Esox Lucius*.** *Journal of Experimental Biology.* 150:321-342
NAL Call No. 442.8 B77
Descriptors: ethology, awareness, performance, behavior, rainbow trout, Northern pike, *Salmo gairdneri, Esox Lucius*

Höglund E, Balm PH, Winberg S (2000) **Skin darkening, a potential social signal in subordinate Arctic charr (*Salvelinus alpinus*): the regulatory role of brain monoamines and pro-opiomelanocortin-derived peptides.** *Journal of Experimental Biology.* 203:1711-1721
NAL Call No. 442.8 B77
Descriptors: ethology, skin pigmentation, behavior, neurobiology, monoamines, pro-opiomelanocortin-derived peptides, Arctic char, *Salvelinus alpinus*

Höjesjö J, Johnsson JI, Axelsson M (1999) **Behavioural and heart rate responses to food limitation and predation risk: an experimental study on rainbow trout.** *Journal of Fish Biology.* 55:1009-1019
NAL Call No. QL614 J68
Descriptors: ethology, behaviour, rainbow trout, *Oncorhynchus mykiss*

Johnsson JI (1997) **Individual recognition affects aggression and dominance relations in rainbow trout, *Oncorhynchus mykiss*.** *Ethology.* 103:267-282
NAL Call No. QL750 E74
Descriptors: ethology, behaviour, awareness, rainbow trout, *Oncorhynchus mykiss*

Johnsson JI, Åkerman A (1998) **Watch and learn: preview of the fighting ability of opponents alter contest behaviour in rainbow trout**. *Animal Behaviour.* 56:771-776
NAL Call No. 410 B77
Descriptors: ethology, behaviour, awareness, rainbow trout, *Oncorhynchus mykiss*

Jones RB (1997) **Fear and distress.** In: *Animal Welfare* (ed. by MC Appleby & BO Hughes), pp. 75-87. CAB International. University Press, Cambridge
NAL Call No. HV4711 A587 1997
Descriptors: ethology, behaviour, awareness, fish, cognition

Lachlan RF, Crooks L, Laland KN (1998) **Who follows whom? Shoaling preferences and social learning of foraging information in guppies**. *Animal Behaviour.* 56:181-190
NAL Call No. 410 B77
Descriptors: ethology, behaviour, awareness, shoaling, guppies

Lamprecht J, Hofer H (1994) **Cooperation among sunfish: Do they have the cognitive abilities?** *Animal Behaviour.* 47(6):1457-1458 ISSN: 0003-3472
NAL Call No. 410 B77
Dugatkin & Wilson (1992) set out to test a common assumption in game theory models of cooperation, namely that animals can remember the outcome of previous interactions with particular individuals and behave with reference to that outcome in future interactions. They concluded that their experiments demonstrated such cognitive abilities in bluegill sunfish, *Lepomis macrochirus*. In this note we ask whether their experiments and results provide

unambiguous support for this conclusion and suggest that this is not the case.
Descriptors: *Lepomis macrochirus*, cognitive ability, cooperativity, learning behavior, freshwater fish, cooperation, intraspecific relationships
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Lankford TE Jr, Targett TE (2001) **Physiological Performance of Young-of-the-Year Atlantic Croakers from Different Atlantic Coast Estuaries: Implications for Stock Structure.** *Transactions of the American Fisheries Society.* 130(3): 367-375
NAL Call No. 414.9 AM3
Geographic variation in life history traits and population dynamics of Atlantic croakers Micropogonias undulatus found north of Cape Hatteras, North Carolina, suggests the possibility of two stocks along the U.S. Atlantic coast. The basis for this variation (i.e., genetic versus ecophenotypic) is unclear. Young-of-the-year Atlantic croakers were collected from three Atlantic coast estuaries (Delaware Bay, Delaware, Cape Fear River, North Carolina, and Indian River Lagoon, Florida) that represent the center and extreme distributional limits of the species' U.S. Atlantic coast range. Intrinsic growth capacity and cold tolerance were measured under common laboratory conditions to test for adaptive genetic variation in these traits. Results were used to evaluate the two stock hypothesis for the Atlantic croaker and to examine the integrity of Cape Hatteras as a possible genetic stock boundary. Growth capacity, feeding rate, growth efficiency, and cold tolerance were similar across geographic locations. Survival curves for Delaware and Florida Atlantic croakers were indistinguishable at each of four low temperatures tested (1, 3, 5, and 7°C), with neither group capable of surviving temperatures of 3°C or less. The suggested lack of adaptive variation we found in these physiological traits supports the hypothesis of a single genetic stock of Atlantic croakers along the Atlantic coast. Although severe winter temperatures may select for fast growth and cold-tolerant genotypes in Northern estuaries, gene flow is apparently sufficient to preclude local genetic adaptation.
Descriptors: estuaries, fish physiology, condition factor, population dynamics, life history, growth, geographical distribution, population genetics, stock identification, biogeography, stock assessment, *Micropogonias undulatus*, ANW, USA, Delaware Bay, ANW, USA, North Carolina, Cape Fear Estuary, ASW, USA, Florida, Indian River Lagoon, USA, Delaware Bay, USA, North Carolina, USA, Florida, Atlantic croaker
ASFA; Copyright © 2003, FAO

Lombardi CM, Hurlbert SH (1996) **Sunfish cognition and pseudoreplication.** *Animal Behaviour.* 52(2):419-422 ISSN: 0003-3472
NAL Call No. 410 B77
In a recent study Dugatkin & Wilson (1992) tested for cognitive abilities in bluegill sunfish. They found that when a focal fish was allowed to forage with different companions, it was able to remember with which ones it had had greatest success and to use this information in future interactions. Moreover, fish seemed to prefer to associate with familiar conspecifics over unfamiliar ones. A number of subsidiary analyses were also reported.
Descriptors: cognitive ability, learning behaviour, freshwater fish, experimental research, *Lepomis macrochirus*, pseudo replication, statistical analysis
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López CJ, Broglio C, Rodríguez F, Thinus-Blanc C, Salas C (2000) **Reversal learning deficit in a spatial task but not in a cued one after telencephalic ablation in goldfish**. *Behavioural Brain Research.* 109:91-98
Descriptors: ethology, awareness, behavior, ablation, goldfish, *Carassius auratus*

López JC, Bingman VP, Rodríguez F, Gómez Y, Salas C (2000) **Dissociation of place and cue learning by telencephalic ablation in goldfish**. *Behavioral Neuroscience.* 114:687-699
NAL Call No. QP351 B45
Descriptors: ethology, awareness, behavior, ablation, goldfish, *Carassius auratus*

Losey Jr GS, Margules L (1974) **Cleaning symbiosis provides a positive reinforcer for fish**. *Science*. 184:179-180
NAL Call No. 470 Sci2
Descriptors: ethology, awareness, behavior, positive reinforcement

Mendl M (1999) **Performing under pressure: stress and cognitive function**. *Applied Animal Behaviour Science.* 65:221-244
NAL Call No. QL750 A6
Descriptors: ethology, awareness, behavior, cognition, stress

Metcalfe NB, Taylor AC, Thorpe JE (1995) **Metabolic rate, social status and life-history strategies in Atlantic salmon.** *Animal Behaviour*. 49:431-436
NAL Call No. 410 B77
Descriptors: ethology, awareness, behavior, physiology, *Atlantic Salmon, Salmo salar*

Miklósi Á, Andrew R J (1999) **Right eye use associated with decision to bite in zebrafish**. *Behavioural Brain Research.* 105:199-205
Descriptors: ethology, awareness, behavior, zebrafish, *Danio rerio, Brachydanio rerio*, eye

Nesse RM (2000) **Is depression an adaptation?** *Archives of General Psychiatry.* 57:14-20
Descriptors: ethology, neurobiology, depression, behavior

O'Connor KI, Metcalfe NB, Taylor AC (1999) **Does darkening signal submission in territorial contests between juvenile Atlantic salmon, *Salmo salar*?** *Animal Behaviour*. 58:1269-1276
NAL Call No. 410 B77
Descriptors: ethology, awareness, behavior, physiology, Atlantic salmon, *Salmo salar*

O'Connor KI, Metcalfe NB, Taylor AC (2000) **Familiarity influences body darkening in territorial disputes between junvenile salmon.** *Animal Behaviour*. 59:1095-1101
NAL Call No. 410 B77
Descriptors: ethology, awareness, behavior, physiology, skin pigmentation, salmon, Salmo

Olla BL, Davis MW (1989) **The role of learning and stress in predator avoidance of hatchery-reared coho salmon (*oncorhynchus kisutch*) juveniles.** *Aquaculture.* 76:209-214
NAL Call No. SH1 A6
Descriptors: ethology, awareness, behavior, physiology, stress, coho salmon, *Oncorylnchus kisutch*

Øverli Ø, Harris CA, Winberg S (1999) **Short-term effects of fights for social dominance and the establishment of dominant-subordinate relationships on brain monoamines and cortisol in raibow trout.** *Brain, Behaviour and Evolution.* 54:263-275
Descriptors: ethology, behavior, neurobiology, monoamines, cortisol, rainbow trout, *Oncorhynchus mykiss*

Øverli Ø, Winberg S, Damsgård B, Jobling M (1998) **Food intake and spotaneous swimming activity in Arctic char (*Salvelinus alpinus*): role of brain serotonergic activity and social interactions**. *Canadian Journal of Zoology*. 76:1366-1370
NAL Call No. 470 C16D
Descriptors: ethology, behaviour, neurobiology, serotonin, Arctic char, *Salvelinus alpinus*

Pitcher TJ (1993) ***Behaviour of Teleost Fishes, 2nd edition***. Chapman & Hall, London
NAL Call No. QL639.3 B44 1993
Descriptors: ethology, behaviour, neurobiology, physiology, awareness, teleost

Popper AN, Carlson TJ (1998) **Application of sound and other stimuli to control fish behavior**. *Transactions of the American Fisheries Society*. 127:673-707
NAL Call No. 414.9 Am3
Descriptors: ethology, behaviour, neurobiology

Schaerer S, Kirschfeld K (2000) **The role of background movement in goldfish vision.** *Journal of Comparative Physiology A*. 186:583-593
NAL Call No. 444.8 Z3
Descriptors: ethology, behaviour, opthamology, neurobiology, goldfish

Schreck CB, Jonsson L, Feist G, Reno P (1995) **Conditioning improves performance of juvenile chinook salmon, *Oncorhynchus tshawytscha*, to transportation stress**. *Aquaculture*. 135:9-110
NAL Call No. SH1 A6
Descriptors: ethology, transport, stress, conditioning, behaviour, Chinook salmon, *Oncorhynchus tshawytscha*

Topál J, Csányi V (1999) **Interactive learning in the paradise fish (*Macopodus opercularis*): an ethological interpretation of the second-order conditioning paradigm**. *Animal Cognition*. 2:97-206
Descriptors: ethology, learning, behaviour, second-order conditioning, paradise fish, *Macopodus opercularis*

Vallortigara G, Rogers LJ, Bisazza A (1999) **Possible evolutionary origins of cognitive brain lateralization.** *Brain Research Reviews*. 30:164-175
Descriptors: cognition, evolution, lateralization, fish

Varner GE (1998) ***In Nature's Interests? Interests, Animal Rights, and Environmental Ethics.*** Envronmental Ethics and Science Policy Series, Oxford University Press, U.S.A.
NAL Call No. GE42 V38 1998
Descriptors: environmental ethics, animal rights, environmentalists, attitude, philosophy of nature

Wainwright PC (1986) **Motor correlates of learning behaviour: feeding on novel prey by pumpkinseed sunfish (*Lepomis gibbosus*).** *Journal of Experimental Biology*. 126:237-247
NAL Call No. 442.8 B77
Descriptors: ethology, behaviour, learning, feeding behaviour, pumpkinseed sunfish, *Lepomis gibbosus*

Webb PW (1986) **Effect of body form and response threshold on the vulnerability of four species of teleost prey attacked by largemouth bass (*Micropterus salmoides*)**. *Canadian Journal of Fisheries and Aquatic Sciences.* 43:763-771
NAL Call No: 442.9 C16J
Descriptors: ethology, behaviour, neurobiology, predator-prey relationship, teleost, largemouth bass, *Micropterus salmoides*

Wood-Gush DGM., Dawkins MS, Ewbank R (1981) *Self-Awareness in Domesticated Animals.* Universities Federation for Animal Welfare. Potters Bar, England.
NAL Call No. QL785 S44 1980
Descriptors: psychology, cognition, ethology, comparative

Zayan R (1991) **The specificity of social stress**. *Behavioural Processes.* 25:81-93
NAL Call No. QL750 B4
Descriptors: ethology, behaviour, stress, physiology, neurobiology

Web Resources:

ASPECTS OF ANIMAL WELFARE AND AQUACULTURE - A Compendium of Selected Literature by Richard D. Moccia and Kristopher P. Chandroo; Aquaculture Centre, University of Guelph, Guelph, Ontario, Canada
http://www.aps.uoguelph.ca/~aquacentre/aec/publications/welfare-bib.html

3.1.4. HEALTH & WELFARE

Alderton D (1988) **Small animals, birds, reptiles and ornamental fish.** *Proceedings. 14th International Conference. Animal Air Transportation Association, Inc. May 8-11, 1988, Amsterdam, The Netherlands.* Animal Air Transportation Association, Inc. Fort Washington, MD 20744, USA. p.131-140
NAL Call No. TL720.7 A5
Descriptors: trade in animals, aquarium fishes, legislation, transport of animals, animal welfare, small mammals

American Humane Association (1985) **Care of fish.** *American Humane Association.* Denver, Colorado. 10 p.
NAL Call No. SF457.C3
Descriptors: fishes, animal welfare

Bjerkaas E, Wall AE, Prapas A (2000) **Screening of farmed sea bass (*Dicentrarchus labrax* L) and sea bream (*Sparus aurata* L) for cataract.** *Bulletin of the European Association of Fish Pathologists. Weymouth. 2(5):180-185*
ISSN: 0108-0288
Screening of sea bass and sea bream for lens changes was carried out on three modern fish farms in Greece at different localisations and farming conditions. The fish were randomly caught with a dip-net for examination and were of different ages and weights. Small cataract changes were diagnosed in 11.9% of the examined sea bream, while only four of 102 examined sea bass (2.9%) showed small cataract changes. Osmotic lens changes were observed in a considerable number of the small sea bass, as well as in some of the larger bass after anaesthesia, while the same effect was not found in the sea bream examined. In addition, ocular changes, including panophthalmitis, rupture of the globe and uveitis were diagnosed in a few fish in poor condition. The results of the study indicate that cataracts may develop in the two species studied, especially in the sea bream, and that the situation should be monitored as Mediterranean fish farming industry is intensified.
Descriptors: eyes, screening, vision, husbandry diseases, *Dicentrarchus labrax, Sparus aurata*, cataracts
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Brannas E, Alanara A, Magnhagen C (2001) **The social behaviour of fish.** Eds: Keeling LJ, Gonyou HW. *Social behaviour in farm animals.* p.275-304. CABI Publishing, Wallingford, UK
ISBN: 0-85199-397-4
Descriptors: aggressive behaviour, animal behaviour, animal physiology, animal welfare, aquaculture, cannibalism, crowding, environmental factors, group interaction, group size, predator prey relationships, social behaviour, social structure, social systems, space requirements, fishes

Braunbeck T (1998) **Cytological alterations in fish hepatocytes following in vivo and in vitro sublethal exposure to xenobiotics--- structural biomarkers of environmental contamination.** (Eds:) Hinton DE, Streit B. *Fish Ecotoxicology.* Basel Schwitzerland Birkhaeuser Verl pp. 61-140

Cytopathological alterations in hepatocytes of fish following exposure to xenobiotic compounds represent a powerful tool to reveal sublethal effects of chemicals and to elucidate underlying modes of action. The present communication reviews the available information about ultrastructural changes in fish liver as well as isolated hepatocytes, whereas the discussion of in vivo effects is primarily focused on data from rainbow trout (*Oncorhynchus mykiss*) and zebrafish (*Danio rerio*), the presentation of in vitro data has been restricted to results from experiments with rainbow trout hepatocytes due to a lack of data from studies with hepatocytes from other species. Both in vivo and in vitro exposure to xenobiotics results in sensitive, selective, and, especially in in vitro experiments, extremely rapid responses of hepatocytes, which, however, may be confounded by internal parameters (species, sex, age, hormonal status) and external parameters (temperature, nutrition, duration of exposure). Thus, transfer of results and conclusions from one experiment to another is usually not possible. Likewise, in vitro results may not necessarily be extrapolated to the situation in intact fish, and effects by acute toxic exposure cannot be translated into sublethal effects. Hepatocellular reactions consist of both unspecific and substance specific effects, in any case, as a syndrome, the complex of all changes induced by a given xenobiotic, is specific. Especially in the lower exposure range, most, if not all, ultrastructural alterations appear to be fully reversible, upon cessation of exposure, restitution of hepatocellular integrity is usually accomplished within a few days. Most early reactions of hepatocytes apparently serve functions within the general adaptation syndrome, which is induced to compensate for the misbalance in organismic homeostasis. Most ultrastructural alterations after sublethal exposure have, therefore, to be classified as indicators of adaptive processes and may be contrasted to irreversible, i.e., degenerative and truly pathological phenomena. These adaptive processes should by definition, not have consequences at higher levels of biological organization, yet, as biomarkers, they are of ecotoxicological relevance. Thus, with regard to their (eco)toxicological significance, components of this nonspecific "general toxicant adaptation syndrome" may serve as early and sensitive warning signals of chemical exposure, whereas more specific changes may be of advanced diagnostic value and may serve as indices for the identification of xenobiotics. Integration of cytopathological alterations into routine aquatic toxicology requires quantification by means of stereological techniques, which make structural data accessible for statistical analysis and comparable with quantitative techniques such as biochemistry and molecular biological methods. Implementation of cytopathological techniques into routine long term investigations with fish gives credit to the principles of animal welfare and protection, since more in depth analysis of internal mechanisms of sublethal chemical contamination in addition to the study of externally overt symptoms of intoxication adds to a refinentent of fish experiments. One step further towards reduction of animal experiments may be achieved by translation of environmental cytopathology to primary hepatocyte cell cultures.
Descriptors: cell culture, sublethal effects, ultrastructure, liver, *Danio rerio, Oncorhynchus mykiss*

Canfield PJ, Quartararo N, Griffin DL, Tsoukalas GN, Cocaro SE. (1994) **Haematological and biochemical reference values for captive Australian snapper, *Pagrus auratus*.** *Journal of Fish Biology.* 44(5):849-856.
NAL Call No. QL614 J68
Reference values for common haematological and biochemical tests were established for 64 captive Australian snapper, *Pagrus auratus* (Bloch & Schneider). Fish were bled from caudal vertebral sinuses after benzocaine anaesthesia. Haematological results were extremely

variable for total leucocytes and types of leucocytes. There was no correlation between leucocytes counts and leucocrit values (R super(2) = 0.37). Thrombocytes were not determined by direct cell counting due to problem of aggregation. Polychromasia was common but did not correlate with reticulocyte counts (R super(2) = 0.08). Biochemical results were extremely variable for creatine kinase and aspartate transaminase but this was considered a direct result of muscle damage during collection.
Descriptors: biochemical composition, hematology, baseline studies, *Pagrus auratus*

Chiba A, Hamaguchi M, Kosaka M, Tokuno T, Asai T, Chichibu S (1990) **Energy metabolism in unrestrained fish with in vivo ^{31}P-NMR.** *Comparative Biochemistry and Physiology.* 96A(2): 253-255
NAL Call No. QP1.C6
We evaluated changes in high energy phosphate metabolism in unrestrained freshwater loaches by in vivo ^{31}P-NMR. When dissolved oxygen was deficient, both an increase in Pi and a decrease in PCr were observed as the loach struggled. After pretreating the fish with an anesthetic agent, we observed the dependence of high energy phosphate metabolism on changes in dissolved oxygen. Under anesthetic (Pi)/(Pi) + (PCr) ratio, an index of metabolic state, was lower than without anesthesia. Decrease in high energy phosphate metabolism in fish during oxygen deficiency was not caused by poor oxygen supply but resulted from struggle movement due to lack of oxygen.
Descriptors: *Cobitis biwae*, animal metabolism, phosphates, freshwater fish

Claireaux G (1999) **Metabolic scope: An indicator of welfare in fish?.** *Copenhagen-Denmark ICES.* International Counc. for the Exploration of the Sea Copenhagen (Denmark) Theme Sess. Health and Welfare of Cultivated Aquatic Animals. Council Meeting of the International Council for the Exploration of the Sea, Stockholm (Sweden), 27 Sep-6 Oct 1999. 1 pp. Compact Disc
Even though environmental factors affecting the relationship between fish and their environment are relatively well identified, documented scientific arguments on their interactions and the dynamics of their effects are scarce. Consequently, to date there exist no unequivocal and easily implemented methods to assess the reactions of an animal to its natural or rearing environment. With regards to the fish-environment interactions, environmental factors are deciding because, (1) they determine the size of fish realised niche, {2) they dictate the general conditions governing the use of this environment, (3) they set the maximum power output that animals will have their disposal to support routine activities. Therefore it ensures, that in order to survive, a fish must continually maintain the appropriate balance between two requirements. The first one is obviously to attain the power output necessary to fulfil the energetic requirements associated with its habitat. The second one is to operate as low as possible from its topmost physiological limits. The maximisation of this functional safety margin between the realisable and the actual metabolic rate is indeed crucial, especially if one considers that the magnitude of that scope provides for natural contingencies, and in fine, sets productivity level (fish farm) and/or mortality risk (natural ecosystem). In this context, we formulated our working hypothesis as follow: a maximised metabolic scope is synonymous with reduced energy budgeting conflicts, increased welfare, and thus with maximised ecosystem/farm yield. As a contribution to the understanding of the fishmilieu interactions, the authors designed an experimental and modelling procedure to analyse the environmental impact on fish metabolic scope. Using this procedure, the authors

then tested the hypothesis that fish do behaviourally optimise their aerobic metabolic capacity. Finally, the authors analysed particular situations in which energy budgeting conflict occurred. (DBO).
Descriptors: fish, animal welfare, metabolism, environmental factors, aquaculture
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Deutsche Veterinarmedizinische Gesellschaft (1978) **Tierschutzgerechte Halterung, Transport und Toten von Susswasserfischen. Tagung der Fachgruppe Tierschutzrecht am 10-11 November 1977 in Munchen [Humane keeping, transport and killing of freshwater fish. Meeting of the Animal Welfare group on 10 - 11th November 1977 in Munich].** *Du und das Tier*. 8(1):8-37 *(In German)*
ISSN: 0341-5759
The 16 papers presented at this meeting are published in this number of the journal. They cover various aspects of animal welfare in relation to fisheries, the good health of fish, stress and the blood picture, pain in the fish, oxygen requirements, temperature and hydrogen, humane killing, and the use of tranquillizers during transport.
Descriptors: fish farming, animal welfare, fisheries, transport of animals
Copyright © 2003, CAB International.

Elliott DG, Pascho RJ, Manning PR, McKibben CL (1994) **Studies on the potential for transmission of *Renibacterium salmoninarum* to chinook salmon (Oncorhynchus tshawytscha) during code-wire tagging procedures.** *International Symposium on Aquatic Animal Health: Program and Abstracts., Univ. of California, School of Veterinary Medicine, Davis, CA (USA).* p. W-17.5
Binary coded-wire tags have been used extensively for more than 30 years for the identification and management of stocks of anadromous salmonid fishes. During a study of the use of brook stock segregation for the control of bacterial kidney disease (BKD) in hatchery-reared spring chinook salmon Oncorhynchus tshawytscha, we observed evidence suggesting that transmission of the causative agent of BKD, *Renibacterium salmoninarum*, might be enhanced by coded-wire tagging procedures. About 4 months after coded-wire tags were implanted in fish from two brood years, we examined histological sections of 14 tissues from each of 120 spring chinook salmon smolts in the study groups. Up to 36 of the R. salmoninarum-infected fish in some groups had focal infections detected only in the snout area near the site of tag implantation. The lesions associated with the infections often resulted in destruction of the olfactory epithelium and supporting tissues. No focal snout infections were observed in fish that were not marked with coded-wire tags. These data suggested that coded-wire tagging procedures can promote the transmission of R. salmoninarum among fish via contaminated tagging needles or by facilitating the entry of pathogens through the injection would. Further field investigations focused on the occurrence of R. salmoninarum contamination on tagging needles, on coded-wire tags, and in the water of anesthetic baths used during the tagging spring chinook salmon.
Descriptors: *Oncorhynchus tshawytscha, Renibacterium salmoninarum*, tagging, fish diseases, bacterial diseases, histology, disease transmission, lesions
ASFA; Copyright © 2003, FAO

Fiskeriforskning (2000) **Institutt for Fiskeri og Haveruksforskning [Annual Report 2000].** *Fiskeriforskning-Norsk-Institutt-for-Fiskeri-og-Haveruksforskning-AS-Norway*. 40 pp *(In Norwegian)*

This annual report summarizes information about Fiskeriforskning and excerpts from the scientific results in 2000 including: product and processing development for Norwegian seafood industry; seafood marketing and industrial economics; biotechnology in development of products from marine raw material; health and welfare of farmed fish; development of feed and technology for new farmed species; stock estimation and ecology of sea mammals, crustaceans, molluscs, coastal resources and flatfishes.
Descriptors: fishery institutions, aquaculture, seafood, biotechnology, research institutions, annual reports, Norway, fish health, fish welfare
ASFA; Copyright © 2003, FAO

HashimotoY, Nishiuchi Y (1983) **Effects of herbicides on aquatic animals**. Eds: Miyamoto J, Kearney PC, Takahashi N, Yoshioka H, MisatoT, Matsunaka S. *Pesticide chemistry, human welfare and the environment. Volume 2. Natural products.* Pergamon Press. Oxford, U.K. p.355-358
NAL Call No. SB951 I562 1982
LC-50 values of 35 herbicides are tabulated for carp, Daphnia, Physa and tadpoles. Trials demonstrating the acute toxicity of PCP and sub-acute toxicity of molinate to carp are discussed. In addition to the haemorrhagic anaemia induced in carp by molinate, vertebral deformities in certain kinds of fish caused by trifluralin or benthiocarb are considered.
Descriptors: Molinate, toxicology, Trifluralin, THIOBENCARB, herbicides, fishes, thiocarbamate herbicides, carbamate pesticides, pesticides, aquatic animals
Copyright © 2003, CAB International.

Institut Natl. de la Recherche Agronomique, Paris; IFREMER, Issy-les-Moulineaux (1999) **Premieres journees de la pisciculture, Nantes, 2-4 novembre 1999: actes [First workshop of the pisciculture, Nantes, 2-4 November 1999: acts].** *Nantes France Ifremer.* 145 pp. *(In French with English Summary)*
This symposium has been organized by many research institutes and partners of the fish sector. The following topics were developed: research and development of this activity, economic analysis, administration, reglementation, quality of the products, the fishes welfare, pathology, environment and nutrition, importance of the professional, and the fish ponds.
Descriptors: conferences, fish culture, economics, legislation, research, experimental, research, quality assurance, biological stress, pathology, nutrition, pond culture, animal welfare
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Jencic V (2000) **Skrb za dobrobit rib[Concern for the fish welfare]**. *Slovenski veterinarski zbornik.* 37(3):163-169 *(In Polish)*
NAL Call No. SF604 L52
Descriptors in English: fishes, animal welfare, behaviour; fish culture, aquaculture, fishing operations, stress, ethics, value, systems, aquaculture

Jones D (1998) **Free captive fish**. *Nature (United Kingdom).* 392(6673):234 ISSN: 0028-0836
NAL Call No. 472 N21
Descriptors: fishing, ecosystem, biochemical oxygen demand, fish, environmental protection, animal welfare, nonhuman, short survey

Kanshik SJ, Luquet P (eds.) (1993) **Fish nutrition in practice**. The 4th International Symposium on Fish Nutrition and Feeding, Biarritz (France), 24-27 Jun 1991. *Colloq. Inra Paris France Institut National De La Recherche Agronomique.* 61. 974 pp.
NAL Call No. S539.7 C6 no.61
In feed-based aquaculture, adequate nutrition practices are decisive in order to be economically competitive as well as to ensure the welfare of the fish. This symposium was held under the auspices of the International Union of Nutrition Sciences. The following topics have been discussed: fish nutrition and reproductive performance; nutrition and health; metabolism and growth; environmental effects; larval and crustacean nutrition; aquaculture and tropical nutrition; and nutrition research and aquaculture development. More than one hundred presentations have stressed the role of nutrition on growth, reproduction and health of species raised in fish farms.
Descriptors: conferences, animal nutrition, feed efficiency, fish culture, aquaculture development, disease resistance, nutrient deficiency, growth, animal metabolism, aquaculture effluents, warm water aquaculture, fish larvae, brood stocks, marine crustaceans, nutritional requirements
ASFA; Copyright © 2003, FAO

Mayes MA, Barron MG (1991) **Aquatic Toxicology and Risk Assessment: Fourteenth Volume.** Proceedings from the 14th ASTM Symposium on Aquatic Toxicology and Risk Assessment, San Francisco, CA (USA), 22-24 Apr 1990. *ASTM Special Technical Publication.* Philadelphia, PA USA American Society For Testing and Materials. 388 pp.
NAL Call No. QH545 W3S95
The objective of the 14th ASTM Symposium on Aquatic Toxicology and Risk Assessment was to provide aquatic toxicologists a forum to share new ideas, to demonstrate the application of old ideas, and to challenge current dogma. To this end, we organized ten sessions that ranged from the specific (Organ System Toxicology and Biomarkers) to the more general (Risk Assessment and Complex Mixtures). The symposium was initiated with a panel discussion on "The Animal Welfare Act: Implications and Predictions in Lower Vertebrate Research." This timely subject was presented from the viewpoint of academia, industry, and governmental regulatory and funding agencies. All agreed that now is the time to become involved in this issue in order to influence future regulations. Traditional environmental effects testing have endpoints that assess real-time exposure-related effects or responses. The session on Biomarkers presented methods/examples that provide an indication of previous and/or current pollutant or stress or exposure. In the session of Organ System Toxicology the contributors explored the basic physiological processes of specific target organs and argue that an understanding of these processes are basic to understanding the mechanism of toxic action of specific chemicals. The use of fish to assess the potential carcinogenicity of xenobiotics was the focus of the Carcinogenesis session. Topics ranged from the complexities of carcinogenesis in the fish liver to the description and validation of fish carcinogenicity models. As regulatory agencies begin to include biological assessments in an effort to meet the objectives of several environmental laws, biomonitoring is becoming more prevalent. Several sessions addressed the methods and strategies for biomonitoring point sources as well as monitoring to assess the effectiveness of bioremediation strategies. The sessions on Toxicity Evaluation and Toxicant Reduction Strategies included topics that focused on the utility and limitations of current test methods, the use of a toxicity index for data extrapolation, the utility of specific species for aquatic testing,and factors that may effect interpretation of the results. The concern about the toxicity of contaminated sediments was addressed in a series of presentations in the session on Sediment Toxicity Assessment.

Both bioaccumulation and toxicity assessment strategies were presented along with considerations of intrinsic characteristics of the sediment that may influence the interpretation of test results.
Descriptors: aquatic environment, toxicology, conferences, hazard assessment, exposure tolerance, environmental monitoring, aquatic environments, risk assessment, fish, animal welfare
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Midtlyng PJ (1997) **Vaccinated fish welfare: protection versus side-effects.** *Developments in Biological Standardization (Switzerland).* 90:371-379. National Centre for Veterinary Contract Research and Commercial Services, Ltd. (VESO AS), Oslo, Norway.
NAL Call No. QR180.3 D4
Active immunisation of fish involves a number of potentially harmful procedures like handling, anaesthesia or injection of more or less toxic substances. Adjuvanted vaccines may cause inflammation, granuloma and pigmentation at the site of injection. Intraperitoneal administration of oil-adjuvanted vaccines to Atlantic salmon pre-smolts has occasionally resulted in impaired growth and reduced carcass quality. The consequences of such vaccination for fish welfare may therefore be questioned. With respect to furunculosis caused by *Aeromonas salmonicida*, scientific data suggest, however, that only oil-adjuvanted vaccines are protective throughout the production cycle of farmed salmon. Data are presented to show that salmonids are highly at risk to epizootics if left unprotected against this or other endemic diseases. A panel of parameters partly adopted from experimental animal medicine is proposed to assess the impact of vaccine side-effects in farmed fish. In intensive salmon aquaculture systems, reduced disease risks are thought to justify the observed level of side-effects following current vaccination practices. For future fish vaccines, reduction of side-effects without compromising long-term protective immunity constitutes a challenging goal.
Descriptors: fishes, vaccination, adjuvants, immunologic, administration, dosage, adverse effects, animal welfare, fish diseases, prevention and control, fisheries, injections, safety, Salmonidae, vaccines

Ribelin WE, Migaki G (1975) ***The Pathology of Fishes***, U of WI Press, Madison, Wisconsin. 1004 pp.
NAL Call No. SH171 P38
Descriptors: fishes, disease, medical, veterinary, pathology, medicine

Ollenschlager B (1978) **Der Einfluss von Temperatur und Wasserstoffionenkonzentration auf den Fisch [Influence of temperature and pH (of water) on fish].** *Du und das Tier.* 8(1):24-25 *(In German)*
ISSN: 0341-5759
Descriptors: animal welfare, environmental temperature, pH, fishes

Poppe TT, Haastein T (1984) **Fisk og dyrevern [Fishes and animal welfare].** *Norsk Veterinaertidsskrift*, 96(3):179-181. *(In Norwegian)*
NAL Call No. 41.8 N81
Descriptors: fishes, animal welfare, fish culture, animal health, animals, aquaculture, aquatic animals, aquatic organisms, foods, vertebrates

Pough FH (1996) **Setting guidelines for the care of reptiles, amphibians and fishes.** *Scientists Center for Animal Welfare (SCAW) newsletter.* 18(1):3-8.

Arizona State University West, Phoenix, AZ.
NAL Call No. QL55.N48
Descriptors: reptiles, amphibia, fishes, animal husbandry, animal welfare, guidelines

Reichenbach-Klinke HH (1987) **Fisch und Naturschutz [Fish and natural protection].** *Tieraerztliche Praxis*. 15(1): 99-106. (*In German*)
NAL Call No. SF603.V4
Descriptors: fishes, fishing operations, transport, stress, slaughtering, environmental effects, fish diseases, pathology, histology, animal welfare, animal health, animals, aquatic animals, aquatic organisms, diseases, disorders, dysregulation, functional disorders, harvesting, injurious factors, methods, processing, vertebrates

Reichenbach-Klinke HH (1978) **Die wesentlichen Parameter fur das Erkennen einer Beeintrachtigung des Wohlbefindens des Fisches [Important values for the recognition of detrimental effects on the well being of fish].** *Du und das Tier.* 8(1):12-14 *(In German)*
ISSN: 0341-5759
Descriptors: stress, transport of animals, animal welfare, fish diseases, fishes

Reynnells RD, Eastwood BR (1998) **Animal welfare issues compendium.** *Poultry Science* 77 (Supplement 1):151
NAL Call No. 47.8 Am33P
Descriptors: animal care, animal husbandry, fish, animal welfare issues, compendium, aquaculture, beef production, conservation, dairy production, hunting, veal production

Sagar Kumar, Shalini Chauhan, Chauhan RRS (1997) **Long-term effect of light on haematology of a fish *Clarias batrachus* (Linn.).** *Uttar Pradesh Journal of Zoology.* 17(1):50-52
ISSN: 0256-971X
The exposure of *Clarias batrachus* to constant light for 24 h resulted in a general elevation in red blood cell count, blood urea, haemoglobin percentage (haematocrit?), blood sugar, bilirubin, alkaline phosphatase and serum transaminase. Levels of cholesterol and serum protein concentration were depleted. All parameters, except for blood urea, returned to pre-exposure levels 5 days later.
Descriptors: light, haematology, blood chemistry, animal welfare, cholesterol, blood sugar, urea, blood proteins, alkaline phosphatase, *Clarius batrachus*

Sauer N, Manz D (1994) **Tierschutztatbestaende bei Fischen [The welfare of fish].** *Tieraerztliche Umschau.* 49(10):653-658 *(In German)*
NAL Call No. 41.8 T445
Welfare legislation applies to fish just as they do to other species. Compared to other pets and domestic animals, fish present a number of differences that present a problem in determining whether the regulations are being observed. These problems are compounded by the diverse uses and management, e.g. breeding, storage and transportation, commercial uses, scientific study and game fishing, to which they are subjected. There is general agreement that fish are capable of suffering and it is therefore immaterial that it has never been established that fish sense pain. In commercial enterprises, causes of suffering can be found as a result of feeding, breeding, over fishing, sorting, mixing of species, transportation and slaughter. Water quality is another source of suffering due to contamination. The major causes for aquarium fish are related to capture, breeding and trade, as well as from errors in

the management and care by aquarists. Game fishing has gained considerable attention with respect to welfare. Examples are presented for all of these diverse activities.
Descriptors: fishes, fishing operations, fish culture, fish feeding, fish ponds, pain, animal welfare, transport of animals, animal health, ornamental fishes, aquaculture, neurophysiology, legal aspects, fish handling, biological stress, sport fishing, hygiene, Teleostei, fish welfare
ASFA; Copyright © 2003, FAO

Sauer N (1993) **Tierschutz bei Fischen [Animmal protection with fishes].** *Fachbereich Veterinaermedizin.* Justus-Liebig-Univ., Giessen (FRG). 290 pp. *(In German)*
In the present dissertation the influence and effects of the human being regarding fishes are analyzed under animal welfare aspects. Respectively a division of the subranges fish cultivating in ponds, cultivation of flowing waters, influences on natural biotopes, angling ornamental fish keeping and fishes as test animals is effected. The foundation are, besides of a multifarious and wide-branching literature, the relevant laws and other regulations, relevant degrees and own experiences of many years.
Descriptors: ornamental fish, fishery protection, ecological balance, legal aspects, spawning, food availability, biological stress, disease control, sport fishing
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Schulz D (1978) **Sauerstoff und Fisch. [Oxygen and fish.].** *Du und das Tier.* 8(1):21-23 *(In German)*
ISSN: 0341-5759
Descriptors: animal welfare, gases, water, oxygen, fishes

Simpson SJ, Raubenheimer D (2001) **A framework for the study of macronutrient intake in fish.** *Aquaculture research.* 32(6):421-432
NAL Call No. SH1.F8
A good understanding of the capabilities of commercially reared fish species to regulate intake of specific macronutrients has potential economic, welfare and environmental benefits. We present a conceptual and experimental framework for studying macronutrient intake in fish. This 'geometric' approach addresses the multidimensional and interactive nature of nutrition. It was developed from work on insect herbivores and has successfully been applied to mammals and birds. The various components of the framework are introduced in simple outlines, and key experimental designs are described for assessing whether or not fish specifically regulate their intake of macronutrients, how they balance over-ingesting some nutrients against undereating others when provided with suboptimal diets, and how they regulate growth post-ingestively.
Descriptors: fishes, experimental design, selectivity, feeding behavior, fish feeding, nutrient uptake, nutrition physiology, growth, literature, fish culture, nutritional requirements, feed efficiency, diets, bioenergetics

Toovey JPG, Lyndon AR, Duffus JH (1999) **Ivermectin inhibits respiration in isolated rainbow trout (*Oncorhynchus mykiss* Walbaum) gill tissue**. *Bulletin of the European Association of Fish Pathology.* 19(4):149-152.
The effect of the anti parasitic compound ivermectin on respiration of isolated rainbow trout gills was investigated. Control (saline only) gills had similar oxygen consumption rates to previous reports for perfused preparations. Ivermectin caused a significant, dose dependent depression of gill oxygen consumption at concentrations of 1.21 mu g/ml and above, with an

EC_{50} of 2.15 mu g/ml. The carrier solvent (propylene glycol) exhibited dose independent inhibition of branchial respiration by 12%. Assuming that these inhibitory effects were cumulative, ivermectin reduced gill respiration by up to 72% at a concentration of 11.2 mu g/ml. In comparison, excess (10 mM) ouabain resulted in a 40% drop in oxygen consumption. It is concluded that further characterisation of ivermectin's impact on fish health, and therefore welfare, is essential if its use for chemotherapeutic purposes in fish is to be licensed.
Descriptors: antiparasitic agents, respiration, oxygen consumption, drugs, disease control, *Oncorhynchus mykiss*

Treasurer J (2002) **Welfare of wrasse**. *Fish Farmer*. 25(6):38-39
NAL Call No. SH151.F574
Although biological control of sea lice by stocking wrasses have environmental benefits, reports from environmental groups have suggested that wrasses were unsuitable because welfare measures are required of them. This article briefly discusses some of the measures to improve the overwintering survival of wrasses.
Descriptors: animal welfare, overwintering, survival, Labridae, Perciformes, Osteichthyes, fishes, vertebrates, Chordata, aquatic organisms, aquatic animals

Wahli T (2002) **Approaches to investigate environmental impacts on fish health.** *Bulletin of the European Association of Fish Pathologists.* 22 (2):126-132
ISSN: 0108-0288
Descriptors: animal health, animal welfare, environmental impact, toxic substances, water pollution, water temperature, brown trout, fishes, *Salmo trutta*, Salmo, Salmonidae, Salmoniformes, aquatic animals

WHO (1990) **Aktuelle Probleme des Tierschutzes. Fortbildungsveranstaltung am 12./13. Oktober 1989 [Current problems of animal welfare. Refresher course held at Hannover on 12-13 October 1989].** *Deutsche Tierarztliche Wochenschrift* 97(4):139-180 *(In German with English summary)*
NAL Call No. 41.8 D482
Individual papers outlined the animal welfare situation in Europe, and welfare problems of pigs, Muscovy ducks, pets, fish, furbearing animals and fallow deer.
Descriptors in English: animal welfare, fish

Witter DJ (1977) **Attitudes Toward Animals and Their Uses: Literature Citations and Animal Welfare Organization Data (Monograph).** *Arizona University, Tucson. Department of Agricultural Economics*. Report Number: AGERS-45, 45 p.
Order this product from NTIS by: phone at 1-800-553-NTIS (U.S. customers); (703)605-6000 (other countries); fax at (703)321-8547; and email at orders@ntis.fedworld.gov.
NAL Call No. HD1775 A6A6
The report contains 486 citations of work about man's feelings toward animals `and their uses, and 80 citations of related social scientific works. The citations are grouped alphabetically by author in four categories: (1) general coverage; (2) animals as recreational and esthetic resources; (3) animals as industrial resources and in biological research; and (4)

related social scientific literature. Additionally, the address, foundation date, membership and staff size, purpose, and periodicals of 141 national organizations concerned with animal welfare are listed.
Descriptors: attitudes, animals, ethics, social psychology, laboratory animals, resources, animal ecology, human behavior, birds, utilization, recreation, sociology, tables (data), statistical data, wildlife, domestic animals, conservation, bibliographies, fishes

Web Resources:

ASPECTS OF ANIMAL WELFARE AND AQUACULTURE -A Compendium of Selected Literature by Richard D. Moccia and Kristopher P. Chandroo; Aquaculture Centre, University of Guelph, Guelph, Ontario, Canada
http://www.aps.uoguelph.ca/~aquacentre/aec/publications/welfare-bib.html

Canadian Council on Animal Care (CCAC)
http://www.ccac.ca
The Canadian Council on Animal Care (CCAC) has posted its second draft of CCAC guidelines on: the care and use of fish in research, testing, and teaching. The draft guidelines and future final guidelines are available at the CCAC webpage (http://www.ccac.ca and http://www.ccac.ca/english/new/newframe.htm).

Fish Products Standards And Methods Manual
Bulletin No. 9 24/03/03
Approved Therapeutants For Aquaculture Use
(This bulletin supersedes and replaces Bulletin no. 8.) The purpose of this bulletin is to inform manual holders of the authorized use of drugs and pesticides in the aquaculture of fish and crustaceans. ....
http://www.inspection.gc.ca/english/anima/fispoi/manman/samnem/smnmalle.pdf

Netvet: Fish resources
http://netvet.wustl.edu/fish.htm

Schreck CB, Moyle PB, editors (1990) **Methods for Fish Biology**. *American Fisheries Society*, Bethesda, Maryland, USA. (http://www.fisheries.org) ISBN: 0-913235-58-X
http://www.fisheries.org/publications/catbooks/mfb.htm

3.1.5. PAIN & STRESS

3.1.5.1. General Topics

Aabel JP, DePauw N, Joyce J (1991) **Stress of Atlantic salmon caused by handling and grading.** *Aquaculture and the Environment. Special Publication, European Aquaculture Society.* 14:1
NAL Call No. SH138.S64
Treatment of fish induces stress to the individuals. Indications of stress can be abnormal behavior like spontaneous migrations towards one part of the cage, increased breathing activity etc., but stress can also lead to increased susceptibility to pathogens and possible higher mortality. Several papers have demonstrated that certain blood parameters can be used as indicators of the physiological condition of the fish. Examining blood samples can give a fairly good impression of the stress level of the fish. Stress in cultured salmon can be associated with different handling procedures like anaesthesia, smoltification, grading, sorting, weighing, but can also be associated with fluctuations in temperature, pH, oxygen, etc. For reasons mentioned in the first chapter it is obviously very important to handle the fish as carefully as possible to minimize the added stress. Through a series of experiments we have tried to quantify the stress added through handling and grading of various sized Atlantic salmon (*Salmo salar*). Both seawater and freshwater have been used. Analyses included concentrations of lactate, glucose, hematocrit, hemoglobin and plasmachloride.
Descriptors: fish physiology, biological stress, mortality causes, handling, fish culture, cultured organisms, hematology, *Salmo salar*
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Adcock PJ, Dando PR (1983) **White muscle lactate and pyruvate concentrations in rested flounder, *Platichthys flesus* and plaice, *Pleuronectes platessa* : A re-evaluation of handling and sampling techniques.** *Journal of the Marine Biological Association of the United Kingdom.* Plymouth. 63(4):897-903
NAL Call No. 442.9 M331
Rapid fixing of skeletal muscle by a "freeze-clamp" technique results in up to a 3-fold lower lactate, a slightly higher pyruvate concentration and a 2- to 4-fold decrease in lactate/pyruvate ratio, to the lowest value yet recorded for fish muscle, when compared with the more usual method of direct immersion in liquid nitrogen. This is attributed to the faster cooling rate of freeze-clamped muscle minimizing "sampling anoxia". Immobilizing fish either by anaesthetic or stunning produces no significant change in metabolite levels. It is concluded that it is relatively easy to handle quiescent flatfish, but light anaesthesia ensures no muscular activity.
Descriptors: muscles, biochemical composition, analytical techniques, biochemistry, metabolites, fixation, *Platichthys flesus, Pleuronectes platessa*, Pisces, *Pleuronectidae, lactate*, pyruvate, freeze-clamp technique
ASFA; Copyright © 2003, FAO

AVMA (2002) **2001 AVMA Animal Welfare Forum, Pain Management**, Hyatt Regency Chicago, Chicago, Illinois, USA, 14 October, 2001. *Journal of the American Veterinary Medical Association.* 221(2):201-237.

NAL Call No. 41.8 AM3
The proceedings cover topics on pain management and welfare implications and medical and case reports in domestic animals, including dogs, cats, laboratory animals, horses and cattle. Topics include evaluation of an ELISA to measure serum thyroxine in dogs and cats, treatment of feline *Haemobartonella felis* with enrofloxacin, effect of fipronil in flea allergic dermatitis-affected cats, acute necrotizing dermatitis and septicaemia in a cat, melanoma in a dog, lufenuron treatment of fungal endometritis in mares, udder cleft dermatitis and sarcoptic mange in a dairy herd, congenital protoporphyria in a calf and surgical removal of a seminoma from a black sea bass (*Centropristis striata*).
Descriptors: animal welfare, blood chemistry, calves, congenital abnormalities, dairy herds, dermatitis, drug therapy, ELISA, endometritis, enrofloxacin, insect growth regulators, insecticides, laboratory animals, melanoma, neoplasms, pain, scabies, surgery, therapy, thyroxine, Perciformes, Osteichthyes, fishes, aquatic organisms, aquatic animals
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Braune HJ, Gronow G (1975) **Temperature as a stressor in *Idus idus* L. (Teleostei).** *Zoologischer Anzeiger.* 194:22-34
NAL Call No. 410 Z7 °
Effects of temp stimuli on substrate contents of the epaxial muscle in *I. idus* (6-7cm) have been studied. Glucose and glucose-6-phosphate content, and the lactate: pyruvate ratio at constant temps were similar in fish acclimated to 15 or 20°C. Both increase and decrease of acclimation temp (AT) from this range generally raised the contents of the substrates mentioned and of the lactate: pyruvate ratio. Pyruvate, however, showed a minimum both at AT 6°C and AT 30°C. The effects of AT 30°C generally exceeded those of AT6°C, indicating a permanent stress caused by the high constant temp. An abrupt transfer in a stage of light anaesthesia (tricaine methanesulfonate, MS 222) from AT 20"C both to 6 and 30°C for 30min caused an increase of the lactate' pyruvate ratio of {approx} 100%. The values remained high for several hrs even after the fish returned to normal at AT20°C, reflecting stress effects. Alternating temps (15 /25°C; 12h/12h) generally raised the substrate contents of *I. dius* (AT 15°C) in the first 10 days. The significant increase of the lactate: pyruvate ration indicates a situation of stress, which is caused mainly by the periodical change of temp and not by the absolute values of the 2 alternating temps. After 10 days alternating temps the values decreased and showed a tendency to reach a constant lower level. The fish seemed to be accustomed to the alternating temp change. The data show that changes in the substrate contents of the epaxial muscle in *I. idus* are an indicator of stress as caused by constant temps and abrupt or alternating temperature changes.
Descriptors: temperature effects, fatigue, musculoskeletal system, *Idus idus*
ASFA; Copyright © 2003, FAO

Carmichael GJ, Tomasso JR, Simco BA, Davis KB (1984) **Characterization and alleviation of stress associated with hauling largemouth bass.** *Transactions of the American Fisheries Society.* 113(6):778-785
NAL Call No. 414.9 AM3
Stress and mortality associated with truck transport of largemouth bass *Micropterus salmoides* were characterized during and after simulated hauling periods of up to 30 hours at a density of 180 g fish/liter of water. Generally, "transported" fish had significantly elevated concentrations of plasma glucose and cortisosteroids and decreased plasma chloride concentrations and osmolality. Significant mortality was associated with hauls of 24 and 30 hours (38% and 83-92%, respectively). Plasma characteristics returned to near-normal 3-28

days after being hauled; recover time generally was related to length of haul and associated mortality. Stress was reduced significantly and mortality was eliminated when fish were treated for diseases, held 72 hours without food before they were loaded, anesthetized before they were loaded, hauled at a cool temperature in physiological concentrations of salts with an antibiotic and a mild anesthetic, and allowed to recover in the same medium less the anesthetic.
Descriptors: fish handling, transportation, mortality causes, biological stress, stocking (organisms), *Micropterus salmoides*, biochemistry, alleviation
ASFA; Copyright © 2003, FAO

Carragher JF, Rees CM (1994) **Primary and secondary stress responses in golden perch, *Macquaria ambigua*.** *Comparative Biochemistry and Physiology, A.* 107A(1):49-56
NAL Call No. QP1.C6
Golden perch (*Macquaria ambigua*), a species of Australian freshwater fish, were subjected to a number of simple stress procedures. Bloodsamples were taken and levels of commonly measured primary and secondary stress response parameters (cortisol, glucose and lactate) were determined. Anaesthesia and exertion of fish prior to bloodsampling affected resting levels of some of the parameters measured. Wild and aquarium-acclimated golden perch had low plasma cortisol levels (< 2 ng/ml). Most fish appeared to adapt well to aquarium conditions, although occasional fish showed indications of being chronically stressed. Golden perch responded quickly to stress (< 5 min), with increased plasma levels of cortisol and lactate. In contrast glucose levels did not increase until at least 10 min after the stress was initiated; by 30 min, however, the typical hyperglycaemic response was observed. Golden perch recover rapidly from acute stress (< 2.5 hr). Golden perch seem to acclimate quickly to conditions of chronic stress.
Descriptors: biological stress, hormones, glucose, blood, acclimation, response analysis, *Macquaria ambigua*
ASFA; Copyright © 2003, FAO

Erdmann C (1999) ***Schmerzempfinden und Leidensfhigkeit bei Fischen. Eine Literaturubersicht. [Ability of Fishes to Feel Pain and to Suffer, a Review].*** Tierarztliche Hoschschule, Hannover Germany. 155 pp. (In German, with English summary)
Descriptors: animal welfare, pain, fishes, aquatic animals

Erikson U, Sigholt T, Seland A (1997) **Handling stress and water quality during live transportation and slaughter of Atlantic salmon (*Salmo salar*).** *Aquaculture* 149(3-4):243-252
NAL Call No. SH1 A6
Atlantic salmon (*Salmo salar*), mean weight 5.1 kg, were transported live for 1.5 h by a well-boat (fish density 125 kg/m^3) from the seacage to a fish processing plant and then kept in the well-boat for 4 h prior to slaughter. Anaerobic white muscle activity due to handling stress during fish loading at the cage, after shipment immediately before slaughter, and after the fish had passed the slaughter line, was evaluated using high-energy phosphates and IMP, the [ATP:IMP] ratio, adenylate energy charge together with pH and redox potential measured directly in the muscle. Water quality parameters, pH, salinity, temperature, dissolved oxygen, carbon dioxide, total carbonate carbon, total alkalinity, ammonia and ammonium were monitored at the cage, during shipment, and in the carbon dioxide anaesthesia tank during commercial fish slaughter. No dramatic effects of handling stress were found, indicating that transport and slaughtering did not have an adverse effect on flesh quality. The results were

explained by the ability of the well-boat to maintain good seawater quality during transport, to a quick bulk netting of the fish from well-boat to the slaughter line and to an efficiently run carbon dioxide anaesthesia-tank that minimised struggling prior to killing.
Descriptors: biological stress, fish handling, transportation, fish culture, cage culture, processing fishery products, aquaculture products, *Salmo salar*

Kestin SC (1994) *Pain and Stress in Fish.* Royal Society for the Prevention of Cruelty to Animals. Amended. Horsham, West Sussex : RSPCA. 36 p.
NAL Call No. SH177.S75K47 1994
Descriptors: fishes, pain, fish culture, animal welfare

Lines JA, Frost AR (1999) **Review of opportunities for low stress and selective control of fish.** *Aquaculture Engineering.* 20(4):211-230
Routine inspection and selective control of livestock is an integral part of animal agriculture benefiting both animal welfare and profitability. Appropriate developments in this field may therefore also be expected within fin fish farming. Equipment enabling some subsurface inspection of fish stocks has recently appeared on the market but this is as yet unmatched by equipment which would enable subsurface selective interaction for the purpose of sampling, further inspection or selective application of remedial measures. Currently available non selective fish control techniques are reviewed and some potential methods for selective control are considered. Since most forms of selective control must rely on fish's response to signals the paper is organised on the basis of the sensory systems that might be used. A brief introduction to each system is given followed by a review of any current control techniques and possibilities for developing selective control systems.
Descriptors: fish inspection, biological stress, sense functions, control

Meyer WF, Cook PA (1996) **An assessment of the use of low-level aerobic swimming in promoting recovery from handling stress in rainbow trout.** *Aquaculture International.* 4(2):169-174
NAL Call No. SH1.A627
Transportation and handling may stress fish (Barton and Peter, 1982; Barton et al., 1986), leading to the undesirable consequence that fish are unloaded from transport units in a stressed condition (Barton et al., 1980; Specker and Schreck, 1980). Characteristically, such stress results in alterations to both behaviour (Sigismondi and Weber, 1988) and physiological state (Mazeaud et al., 1977), which may give rise to transport mortality (Wedemeyer, 1976). Consequently, economic and ethical considerations have prompted numerous studies into methods that reduce transport stress (e.g. Wedemeyer, 1972; Barton and Peter, 1982; Carmichael et al., 1984; Robertson et al., 1988) and the manipulation of the transport water osmolality, cold water transport, and anaesthetic treatment prior to transport, have shown some success. All of the aforementioned methods of fish transport reduce the magnitude of transport stress, but little work has focused on promoting stress recovery. Since the initial loading of fish into the transport container is the most stressful component of transport (Miles et al., 1974; Specker and Schreck, 1980), we were curious to know if the recovery process could be initiated during transport itself, leading to the arrival of fish in a less stressed condition. It was examined whether low-level aerobic swimming following handling stress would hasten stress recovery in rainbow trout (*Oncorhynchus mykiss*) over that of unswum fish.

Descriptors: aquaculture techniques, biological stress, freshwater fish, swimming, *Oncorhynchus mykiss*
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Moberg GP, Mench JA (Eds) (2000) ***The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare.*** 377 pp. CABI Publishing Wallingford, UK
NAL Call No. QP82.2 S8 B55 2000
This book brings together a range of scientific perspectives from biomedical research on stress and welfare, and assesses new approaches to conceptualizing and alleviating stress. While much of the focus in on conventional farm animals, there is also consideration of fishes, laboratory animals and zoo animals. The 30 contributors include leading authorities from North America, Europe, New Zealand and Australia. This book is invaluable for advanced students and researchers in animal behaviour, animal welfare, animal production, veterinary medicine and applied psychology. For more information see the CABI Publishing online bookshop (http://www.cabi.org/Bookshop/).
Descriptors: stress, biology, animal welfare, animal behaviour
Copyright © 2003, CAB International.

Muiswinkel WB van (1999) **The interaction between immune competence and stress responses in relation to fish health problems**. *Fourth Symposium on Diseases in Asian Aquaculture: Aquatic Animal Health for Sustainability November 22 26, 1999, Cebu International Convention Center, Waterfront Cebu City Hotel, Cebu City, Philippines.* Note: (Book of abstracts.)
NAL Call No. SH171 S96
It is known that severe and chronic stress is unavoidable during standard procedures in aquaculture. These conditions may lead to acute mortalities or losses caused by diseases. Improving the resistance to disease and/or stress by genetic means is an attractive approach to reduce these widespread welfare problems in aquaculture. However, genetic selection for one type of response may affect the other, due to the delicate balance between the neuroendocrine system and the immune system. To our knowledge these possible negative effects of selection have not been investigated in fish. Recent results from a multidisciplinary research program (supported by the Dutch SLW/LNV priority program) will be reviewed. The program consists of three closely related and interdependent projects aimed at the analysis of specific carp (*Cyprinus carpio*) strains selected for either stress response or immune competence for the following aspects: 1) The physiological responses to a stressor (crowding), which is typical for an aquaculture situation (Fish Culture & Fisheries Group, Wageningen Agricultural University, NL); 2) The modulating effect of a stressor on the genetically determined differences in immune responsiveness (Cell Biology & Immunology Group, Wageningen Agricultural University, NL); 3) The quantitative analysis of the stress response and of immuno-neuro-endocrine interactions (Dept of Animal Physiology, University of Nijmegen, NL).
Descriptors: fish culture, immunity, genetics, selective breeding, biological stress, animal physiology, husbandry diseases, disease resistance, *Cyprinus carpio*
ASFA; Copyright © 2003, FAO

Oidtmann B, Hoffmann RW (2001) **Schmerzen und Leiden bei Fischen[Pain and suffering in fish].** *Berliner und Munchener tierarztliche Wochenschrift.* 114(7-8): 277-82. (In German with English summary)
NAL Call No. 41.8 B45

The question on the capability of fish to feel pain and of suffering are still subject of discussion nowadays. In the article presented, the information available in the literature to date is summarised. Based on this knowledge, the conclusion is drawn that fish are capable of feeling pain and that they are able to suffer in the sense of the word as used in the German animal welfare law. (66 Refs.)
Descriptors: fishes, physiology, pain, veterinary, animal welfare, legislation and jurisprudence, autonomic nervous system, physiology, Germany, pain prevention and control, stress

Peters G (1988) **Schmerz und Stress bei Fischen. [Stress and pain in fish.]** *Deutsche Tieraerztliche Wochenschrift*. 95(2):60-63. (In German with English summary)
ISSN: 0341-6593
Descriptors: fishes, stress, pain, animal welfare, animal health, animals, aquatic animals, aquatic organisms, disorders, dysregulation, functional disorders, injurious factors, physiological, functions

Rose JD (2002) **The neurobehavioral nature of fishes and the question of awareness and pain.** *Reviews in Fisheries Science*. 10(1):1-38.
NAL Call No. SH1.R425
This review examines the neurobehavioral nature of fishes and addresses the question of whether fishes are capable of experiencing pain and suffering. The detrimental effects of anthropomorphic thinking and the importance of an evolutionary perspective for understanding the neurobehavioral differences between fishes and humans are discussed. The differences in central nervous system structure that underlie basic neurobehavioral differences between fishes and humans are described. The literature on the neural basis of consciousness and of pain is reviewed, showing that: (1) behavioral responses to noxious stimuli are separate from the psychological experience of pain, (2) awareness of pain in humans depends on functions of specific regions of cerebral cortex, and (3) fishes lack these essential brain regions or any functional equivalent, making it untenable that they can experience pain. Because the experience of fear, similar to pain, depends on cerebral cortical structures that are absent from fish brains, it is concluded that awareness of fear is impossible for fishes. Although it is implausible that fishes can experience pain or emotions, they display robust, nonconscious, neuroendocrine, and physiological stress responses to noxious stimuli. Thus, avoidance of potentially injurious stress responses is an important issue in considerations about the welfare of fishes.
Descriptors: pain, nociception, stress, awareness, anthropomorphism, neurophysiology, literature reviews, behavioural responses, biological stress, nature conservation
http://www.news-press.com/multimedia/documents/fishpain/fishpain.pdf

Schreck CB (2000) **Accumulation and long-term effects of stress in fish.** (Eds:) Moberg GP, Mench JA. *The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare*. CABI Publishing, Wallingford, UK. p.147-158.
NAL Call No. QP82.2 S8 B55 2000
Descriptors: stress, stress response, physiology, performance, fishes, aquatic animals

Wells RMG, Tetens V, Devries AL (1984) **Recovery from stress following capture and anaesthesia of Antarctic fish: Haematology and blood chemistry.** *Journal of Fish Biology*. 25(5):567-576
NAL Call No. QL614 J68

Qualitative changes in haematology and blood chemistry of the giant Antarctic cod, *Dissostichus mawsoni*, were followed during recovery from the stresses of capture and cannulation under MS 222 anaesthesia. Cannulation with anaesthesia resulted in a transient rise in haematocrit and haemoglobin concentration, and a fall in blood pH. These changes returned to stable values 8-24 h later. Blood lactate and mean corpuscular haemoglobin concentration remained near to resting values. Experiments with the smaller species, *Pagothenia borchgrevinki*, indicated that erythrocyte swelling, elevated blood lactate, and changes in ATP concentration were delayed manifestations of severe agitational stress. The significance of these findings is discussed in relation to oxygen transport in fish having low metabolic rates, and also in relation to widely used techniques for sampling blood.
Descriptors: hematology, biological stress, *Dissostichus mawsoni, Pagothenia borchgrevinki*

Web Resources:

Aspects of Animal Welfare and Aquaculture -A Compendium of Selected Literature by Richard D. Moccia and Kristopher P. Chandroo; Aquaculture Centre, University of Guelph, Guelph, Ontario, Canada
http://www.aps.uoguelph.ca/~aquacentre/aec/publications/welfare-bib.html

Do Fish Feel Pain?
Dr. James D. Rose
http://www.cotrout.org/do_fish_feel_pain.htm
http://www.anglingmatters.com/DrRoseReport.pdf

Pain in Fish
http://www.vet.ed.ac.uk/animalwelfare/Fish%20pain/Contents.htm

3.1.5.2. Anatomy, Neurophysiology & Pharmacology Studies

Allen JL, Dawson VK, Hunn JB (1999) **Biotransformation of selected chemicals by fish.** Presented at: 176. Meet. American Chemical Society, Pesticide Chemistry Division; Miami Beach, FL (USA); 11 Sep 1978. *In: Pesticide and xenobiotic metabolism in aquatic organisms. Based on a symposium sponsored by the Division of Pesticide Chemistry at the 176th Meeting of the American Chemical Society, Miami Beach, Florida, September 11-17, 1978. No. 99.* Publ. by: American Chemical Society; Washington, DC (USA)., 1979., p. 121-129., ACS Symp. Ser.
NAL Call No. QD1 A45 No. 99
Biotransformation of selected chemicals by freshwater fish is accomplished through a diversity of biochemical pathways. Biliary and renal excretion of glucuronide conjugates of two lampricides, 3-trifluoromethyl-4-nitrophenol (TFM) and 2',5'-dichloro-4'-nitrosalicylanilide (Bayer 73), have been demonstrated. Glucuronide conjugation has also been demonstrated with the fish anesthetic, 2-amino-4-phenylthiazole (Piscaine). Preliminary studies have indicated that fish are capable of hydrolyzing Bayer 73 to two fragments, 5-chloro-salicylic acid and 2-chloro-4-nitroaniline. Hydrolysis of the ester linkage of methane sulfonate of m-aminobenzoic acid ethyl ester (MS-222) to form m-aminobenzoic acid has been shown in freshwater and saltwater fish. Amino groups in MS-222 and Piscaine are subject to N-acetylation. Most of the acid metabolites of the fish anesthetics are excreted renally. Dealkylation of a substituted amine was shown by the stepwise deethylation of dinitramine (NSUP-3,NSUP-3 -diethyl-2,4-dinitro-6-trifluoromethyl-m-phenylenediamine) in carp (*Cyprinus carpio*). Fish are also capable of biotransformation involving substitution; fish exposed to Thanite (isobornyl thiocyanoacetate) apparently release cyanide by substituting a methyl group to form isobornyl- -(methylthio)acetate.
Descriptors: chemical pollutants, pollution effects, *Cyprinus carpio*, Cyprinidae, Pisces
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Allen JL, Luhning CW, Harman PD (1972) **Residues of MS 222 in Northern pike, muskellunge and walleye.** *Technical Paper. Bureau of Sport Fisheries and Wildlife. US Fish and Wildlife Service. Washington DC.* 45:3-8
Residues of MS-222 (tricaine methanesulfonate) in muscle tissue of Northern pike, muskellunge, and walleye following anesthesia were measured by a modified Bratton-Marshall colorimetric method and confirmed by TLC. The residues dissipate rapidly from the muscle when fish are withdrawn from the anesthetic and are near the background readings of the controls within 24 hours.
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Descriptors: MS 222, Northern pike, muskellunge, walleye, anesthetic

Allen JL, Luhnung ChW, Harman PD (1970) **Identification of MS 222 residues in selected fish tissues by thin layer chromatography.** *Investigations in Fish Control.* 41:1-7
NAL Call No. SH157.7 I58
MS-22, a commonly used fish anesthetic, reacts with the Bratton-Marshall reagents to form a winered dye. Residues of MS-222 determined by this reaction are not distinguished from other primary aromatic amines. TLC was used to identify MS-222 in the presence of

background primary aromatic amines in fish muscle, brain, and blood. This method, in which the Bratton-Marshall reaction is used to visualize the spots, gave both the specificity of the Bratton-Marshall reaction for primary aromatic amines and the Rf of MS-222 as tools for identification of the residus. Recoveries of 25 to 60% were obtained in muscle samples spiked with 2 to 10 ppm of MS-222. Quantitative estimation was difficult in samples spiked with 2 ppm or less, but presence of MS-222 residues could be confirmed in samples spiked with as little as 0.2 ppm. Since the meta-aminobenzoate ester can be identified at these concentrations, this should be a useful ancillary or confirmatory method for determining the rate of disappearance of drug residues in fish flesh and obtaining data for clearance and registration of the anesthetic with the Food and Drug Administration.
Descriptors: MS-222, fish, tissues, anesthesia, analgesia, concentration, residues, chromatography,
ASFA; Copyright © 2003, FAO

Beaumont MW, Butler PJ, Taylor EW (1995) **Plasma ammonia concentration in brown trout in soft acidic water and its relationship to decreased swimming performance.** *Journal of Experimental Biology.* 198(10):2213-2220
NAL Call No. 442.8 B77
Adult brown trout (300-600 g) were acclimated for 2 weeks to an artificial soft water (Ca^{2+}, 50 mu $moll^{-1}$) and maintained at either 5°C (October to March) or 15°C (May to August). Following insertion of a cannula into the dorsal aorta under MS-222 anaesthesia and a recovery period of 2 days, the fish were exposed to a 4 day episode of sub-lethal copper levels at pH5 or kept at control conditions of pH7 without copper. The copper concentrations had been predetermined by toxicity testing and were approximately 0.47 mu $moll^{-1}$ at 5°C and 0.08 mu $moll^{-1}$ at 15°C. At 5°C, a group of fish was also exposed to approximately 0.08 mu $moll^{-1}$ copper at pH5. Plasma total ammonia (T_{amm}) concentration was significantly elevated by exposure to copper and pH5. In resting trout exposed to the appropriate sub-lethal copper concentration at pH5, T_{amm} was six and 7.5 times greater at 5 and 15°C, respectively, than those of control trout at the respective temperatures. Although unconfirmed, an elevation of ammonia production alone seems unlikely to account for such substantial increases. From previous studies, there is little evidence of impairment of respiratory gas exchange in trout exposed to these copper concentrations and yet, in the acidic test waters, the gradient of NH_3 partial pressure between fish and water was 5.5-6 times greater than that under control conditions. Swimming performance determined by the critical swimming speed (U_{crit}) was reduced by copper and acid exposure, and a significant relationship existed between U_{crit} and the plasma ammonia concentration of exercised trout. Ammonium ions influence several key enzymes involved in energy metabolism, and elevated ammonia levels might, therefore, reduce the capacity of muscle to exercise. Alternatively, ammonia may have affected the nervous coordination of exercise either centrally or by disrupting peripheral motor innervation.
Descriptors: swimming, copper, pH effects, ammonia, haematology, pollution effects, hypoxia, *Salmo trutta*, pH, hematology, toxicity testing, trout, sublethal effects, hydrogen ion concentration, toxicity, water pollution effects
ASFA; Copyright © 2003, FAO

Belanger SE, Schurr K, Allen DJ, Gohara AF (1986) **Effects of chrysotile asbestos on coho salmon and green sunfish: Evidence of behavioral and pathological stress.** *Environmental Research.* 39(1):74-85

NAL Call No. RA565 A1E5
The effects of chrysotile asbestos on larval coho salmon (*Oncorhynchus kisutch*) and juvenile green sunfish (*Lepomis cyanellus*) were investigated at levels approximating those reported in the Great Lakes basin (10^6 fibers/liter). Behavioral stress effects, such as loss of rheotaxic position and balance, were observed in salmon exposed at 3.0 x 10^6 fibers/liter and in sunfish exposed at 1.5 and 3.0 x 10^6 fibers/liter. Coho larvae at 1.5 x 10^6 fibers/liter were significantly more susceptible to an anesthetic stress test, becoming ataxic and losing equilibrium faster than control cohorts. Two of 106 larvae exposed at 3.0 x 10^6 fibers/liter developed tumorous swellings and three additional fish developed coelomic distentions. Cytological examination of ventral epidermal tissue revealed cellular histolysis, and evidence by transmission electron microscopy confirmed the presence of asbestos in the salmon larvae. Distortion of the lateral line region in asbestos-treated coho salmon was linked to behavioral and orientational aberrations.
Descriptors: asbestos, behavior, pathology, toxicity, *Oncorhynchus kisutch, Lepomis cyanellus*, effects on
ASFA; Copyright © 2003, FAO

Benoit E, Laurent D, Mattei C, Legrand AM, Molgo J (2000) **Reversal of Pacific ciguatoxin-1B effects on myelinated axons by agents used in ciguatera treatment.** *First meeting on Ichtyology in France, RIF 2000. Cybium – Paris.* 24(3S):33-40
ISSN: 1399-0974
Ciguatera fish poisoning is a distinctive form of ichthyosarcotoxism characterised mainly by gastrointestinal and neurological disturbances. The ciguatoxins, responsible for this poisoning, are complex polyethers produced by toxic strains of the dinoflagellate Gambierdiscus toxicus. These toxins are increased to dangerous levels for man during their transmission through herbivorous and carnivorous fish, various species being contaminated. The known molecular target of ciguatoxins is the voltagegated Na+ channel. During the action of these toxins, the permanent opening of channels, at the resting membrane potential, produces a continuons entry of Na+ ions in excitable tells causing a marked increase in membrane excitability and in cellular volume. To precise the neurocellular bases of the efficacy of some agents used in clinical and traditional treatments of ciguatera, their effects were studied on frog myelinated axons exposed to Pacific ciguatoxin-1B (CTX-1B). During the action of this toxin, the increase in axonal volume and membrane excitability was reversed by lidocaine (a local anaesthetic), by CaCl2 and by hyperosmotic external solutions (containing D-mannitol, sucrose or tetramethylammonium chloride). The CTX-1B-induced hyperexcitability of the membrane was also reversed by extracts of *Argusia argentea* leaves or *Davallia solida* rhizomes, used traditionally in New-Caledonia. It is concluded that the various agents studied are able to counteract the neurocellular effects of CTX-1B in myelinated axons. These results are of particular interest since they provide a scientific basis to understand the beneficial action of therapeutic agents used in the treatment of ciguatera fish poisoning.
Descriptors: ciguatoxin, fish poisoning, ions, therapy
ASFA; Copyright © 2003, FAO

Benzer TI, Raftery MA (1972) **Partial characterization of a tetrodotoxin-binding component from nerve membrane.** *Proceedings of the National Academy of Sciences, USA.* 69(12):3634-3637
NAL Call No. 500 N21P
Tecrodotoxin from Japanese puffer fish has been labeled with tritium and purified from the

crude mixture obtained. The interaction between the purified [^{3}H]tetrodotoxin and membrane suspensions from the olfactory nerve of long-nosed garfish has been investigated by equilibrium dialysis. Tetrodotoxin binds to membrane suspensions with a dissociation constant K_D=8.3nM. The nerve preparation binds 42 pmol of [^{3}H]tetrodotoxin/g of wet tissue at saturating toxin concentrations. With various hydrolic enzymes, the binding component is shown to be a protein embedded in a phospholipid environment. The binding is inhibited below pH 4.0 and is not stable towards heat. Tetrodotoxin binding is not inhibited by the local anesthetic, procaine.
Descriptors: tetrodotoxin, puffer fish, nerve tissue

Bernstein JJ (1970) **Anatomy and physiology of the central nervous system**. In: Hoar, W.S. and Randall, D.J. (eds) *Fish Physiology, Vol. IV*, Academic Press, New York, p. 1-78
Descriptors: fish, anatomy, physiology, CNS, central nervous sytem, neurobiology

Bradford Jr. MR (1995) **Comparative aspects of forebrain organization in the ray-finned fishes: touchstones or not?** *Brain, Behavior and Evolution.* 46:259-274
Descriptors: neurobiology, CNS, comparative evolution, ray-finned fish

Broderius SJ, Kahl M (1985) **Acute toxicity of organic chemical mixtures to the fathead minnow.** *Aquatic Toxicology.* 6(4):307-322
NAL Call No. QH541.5.W3A6
The acute joint toxicity of industrial organic chemicals to the fathead minnow (*Pimephales promelas*) was determined for binary and equitoxic multiple chemical mixtures. Results from binary tests were used to define isobole diagrams. The degree of joint toxic action was determined among 27 chemicals from seven different chemical classes. The slopes of the acute concentration response relationships were quite similar for all test chemicals. This suggests that the mode of acute toxic action for these chemicals is alike though it may not be identical. Intoxication signs of fish exposed to nearly all test chemicals were also similar and indicative of an anesthetic like effect. The results of isobole diagrams for binary mixtures, with 1-octanol as the reference chemical, demonstrated a near concentration additive acute joint action over a wide range of mixture ratios, for each chemical from 7 different classes.
Descriptors: organic compounds, synergism, toxicity, chemical pollutants, *Pimephales promelas*, acute toxicity, mixtures

Brown EAB, Franklin JE, Pratt E, Trams EG (1972) **Contributions to the pharmacology of quinaldine (uptake and distribution in the shark and comparative studies).** *Comparative Biochemistry and Physiology.* 42(1A):223-231
NAL Call No. QP1.C6
(1) Comparative toxicity and anaesthetic conc of quinaldine (2-methylquinoline), 9 related quinolines and 2-methylpyridine were studied in mice and several spp of fish. The compds were administered as methanesulfonate salts. (2) The absorption and excretion rates of quinaldine were similar in fish and the drug was excreted unchanged. (3) The anaesthetic conc in sea water for 2-, 6-, 7-, and 8- methylquinolines in fish was similar, 0.05-0.15 mM. (4) The LD50 values for mice were of the same order of magnitude for all quinolines studied, i.e. 0.5-3 m-moles/kg, i.p.
Descriptors: quinaldine, shark, anesthetic, fish, LD50

Butler AB (2000) **Topography and topology of the teleost telencephalon: a paradox resolved**. *Neuroscience Letters*. 293:95-98
NAL Call No. QP351 N3
Descriptors: teleost, telencephalon, fish, neurobiology

Carruth LL, Jones RE, Norris DO (2000) **Cell density and intracellular translocation of glucocorticoid receptor-immunoreactive neurons in the Kokanee salmon (*Oncorhynchus nerka kennerlyi*) brain, with an emphasis on the olfactory system.** *General and Comparative Endocrinology*. 117:66-76
NAL Call No. 444.8 G28
Descriptors: neurobiology, glucocorticoid receptor-immunoreactive neurons, Kokanee salmon, *Oncorhynchus nerka kenerlyi*

Cameron AA, Snow PJ, Plenderleith MB (1990) **Organization of the spinal cord in four species of elasmobranch fish: Cytoarchitecture and distribution of serotonin and selected neuropeptides.** *Journal of Comparative Neurology*. 297:201-218
NAL Call No. QP351 J68
Descriptors: neurobiology, neurology, CNS, elasmobranch, shark, serotonin, neuropeptides

Coggeshall RE, Leonard RB, Applebaum ML, Willis WD (1978) **Organization of peripheral nerves and spinal roots of the Atlantic stingray, *Dasyatis sabina*.** *Journal of Neurophysiology*. 41:97-107
Descriptors: neurology, peripheral nervous system, Atlantic stingray, *Dasyatis sabina*

Corrêa SAL, Corrêa FMA, Hoffmann A (1998) **Stereotaxic atlas of the telencephalon of the weekly electric fish *Gymnotus carapo*.** *Journal of Neuroscience Methods* 84:93-100
Descriptors: neurobiology, telencephalon, CNS, electric fish, *Gymnotus carapo*

Davis RE, Kassel J (1983) **Behavioral functions of the teleostean telencephalon**. In: *Fish Neurobiology. Volume 2: Higher Brain Areas and Functions* (ed. by R.E. Davis & R.G. Northcutt), pp. 238-263. University of Michigan Press, Ann Arbor
Descriptors: teleost, telencephalon, neurobiology, behaviour

Demski LS (1983) **Behavioural effects of electrical stimulation of the brain**. In: *Fish Neurobiology. Volume 2: Higher Brain Areas and Functions* (ed. by R.E. Davis and R.G. Northcutt), pp. 317-359. University of Michigan Press, Ann Arbor
Descriptors: neurobiology, CNS, brain, behaviour, electrical stimulation

Devor A (2000) **Is the cerebellum like cerebellar-like structures?** *Brain Research Reviews*. 34:149-156
Descriptors: neurobiology, cerebellum, telencephalon

Di Marco P, McKenzie DJ, Mandich A, Bronzi P, Cataldi E, Cataudella S (1999) **Influence of sampling conditions on blood chemistry values of Adriatic sturgeon *Acipenser naccarii* (Bonaparte, 1836).** Proceedings of the 3. International Symposium on Sturgeon, Piacenza, Italy, July 8-11, 1997. *Journal of applied ichthyology / Zeitschrift fur angewandte Ichthyologie*. Hamburg, Berlin. 15(4-5):73-77

NAL Call No. QL614.Z44
Data on the blood chemistry of a chondrostean fish, the Adriatic sturgeon (*Acipenser naccarii*), are reported as measured with different sampling procedures, and as related to rearing conditions and age. Serum cortisol, glucose, osmolality, Na^{+}, Cl^{-}, Ca^{2+} and total protein concentrations were measured. Reference values for the blood chemistry of farmed sturgeon were measured on samples from resting undisturbed animals collected via a thronic indwelling catheter in the dorsal aorta that was implanted under anaesthesia. Following 24 h recovery from catheterization, serum cortisol, glucose and osmolality levels were 9.4 ng/ml, 58.8 mg/dl and 261.4 mOsm/kg, respectively. Furthermore, blood samples collected with the chronic indwelling catheters indicated that the surgical procedure of cannulation caused a stress response, with physiological changes that followed a pattern like that described in teleosts. Cortisol, glucose and osmolality were more sensitive to stress than the other variables measured. Sampling by cardiac puncture tended to be associated with elevated serum cortisol levels in older, larger sturgeon, but not in young fish. Greater capture, confinement and handling stress in older, larger, sturgeon may have been responsible for this and other age-related differences in blood chemistry values measured following cardiac puncture. Within the same age class, both rearing conditions and temperature affected cortisol, sodium and total protein concentrations significantly. Anaesthesia did not appear to reduce the degree of stress associated with cardiac puncture but altered serum ion concentrations.
Descriptors: haematology, rearing, environmental conditions, biological stress, *Acipenser naccarii*
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Dorsen M (1974) **Production of anti-dinitrophenol precipitating antibody in rainbow trout alevins (*Salmo gairdneri*) immunised at one month old.** *Comptes Rendus. Academie des Sciences. Serie D.* 278(24):3151-3152
Month-old rainbow trout alevins, of mean weight 0.15g, were immunised by abdominal injection of dinitrophenol (DNP) combined with keyhole-limpet haemocyanin, under MS-222 anaesthesia. Three months later (at mean body weight 3.0g) serum tested by Ouchterlony's gel diffusion technique with DNP-haemocyanin gave a precipitation reaction in 10 out of the 28 fish. Thus in an alevin at one month, i. e. before feeding begins, immune competence may already have developed.
Descriptors: immunology, *Oncorhynchus mykiss*
ASFA; Copyright © 2003, FAO

Drapeau P, Legendre P (2001) **Neuromuscular transmission on the rebound.** *Receptors and Channels.* 7(6):491-496
ISSN: 1060-6823
Recent work at the zebrafish neuromuscular junction (NMJ) has shown that positively charged acetylcholine (ACh), at the high concentrations reached in the cleft during neuromuscular transmission, blocks acetylcholine receptors (AChRs) as soon as they open. Thus after two ACh molecules bind and open the channel, a third molecule enters and blocks the pore at a site resembling that for block by local anesthetics, suggesting that ACh is the endogenous anesthetic of the NMJ. Recovery from open channel block results in a rebound synaptic current only after ACh is cleared from the cleft. Kinetic modeling of other AChRs suggests that a rebound current is generated at all vertebrate NMJs, from fish to frogs to mammals. Open channel block prolongs the current at fast zebrafish NMJs in order to more effectively spread charge along the fibers, akin to multiple central synapses spread over

dendrites. Together these findings indicate the need for a fundamental revision of current thinking about neuromuscular transmission at many levels, including channel structure, function and pharmacology.
Descriptors: nerves, muscles, neurotransmitters, electrophysiology, *Danio rerio*, zebra danio

Echteler SM, Saidel WM (1981) **Forebrain connections in the goldfish support telencephalic homologies with land vertebrates.** *Science.* 212:683-684
NAL Call No. 470 Sci2
Descriptors: neurobiology, CNS, goldfish, telencephalon, cerebellum

Ehrensing RH, Michell GF, Kastin AJ (1982) **Similar antagonism of morphine analgesia by MIF-1 and naloxone in *Carassius auratus*.** *Pharmacology Biochemistry and Behavior.* 17:757-761
NAL Call No. QP901 P4
Descriptors: morphine, biochemistry, neurobiology, MIF-1, naloxone, *Carassius auratus*

Fibiger HC, Phillips AG (1986) **Reward, motivation, cognition: psychobiology of mesotelencephalic dopamine systems.** In: *Handbook of Physiology. Volume 4; The Nervous System* (e.d by V.B. Mountcastle, F.E. Bloom & S.R. Geiger), pp. 647-675. American Physiological Society. Bethesda, Maryland
Descriptors: neurobiology, physiology, psychobiology, mesotelencephalic dopamine system

Finger TE (1980) **Nonolfactory sensory pathway to the telencephalon in a teleost fish**. *Science.* 210:671-673
NAL Call No. 470 Sci2
Descriptors: neurobiology, telencephalon, nonolfactory sensation, teleost

Finger TE (1983) **Organization of the teleost cerebellum.** In: *Fish Neurobiology. Volume 1: Brain Stem and Sense Organs* (ed. by R.G. Northcutt & R.E. Davis) pp.261-284. University of Michigan Press, Ann Arbor
Descriptors: neurobiology, teleost, telencephalon, cerebellum

Fuller JD, Scott DBC, Fraser R (1976) **The reproductive cycle of *Coregonus lavaretus* (L) in Loch Lomond, Scotland, in relation to seasonal changes in plasma cortisol concentration.** *Journal of Fish Biology.* 9(2):105-117
NAL Call No. QL614 J68
The reproductive cycle of *C. lavaretus* in Loch Lomond, Scotland, was investigated by monthly sampling. Spawning takes place during the first 3 weeks of Jan, on offshore gravel banks. Males congregate on the spawning-grounds throughout the spawning period, while ripe female cruise in unisexual shoals in deeper water. Individual female migrate to the spawning-grounds as ovulation takes place, spawn, and return to deep water. After spawning, the gonadosomatic ratio and somatic condition factor of both sexes decrease until June or July. Gonad recrudescence occurs in male between July and Oct, and in female between July and Dec. The somatic condition factor of both sexes rises from its minimum in June or July to its maximum in Sept. A competitive protein binding assay was used on determine cortisol levels in 0.1ml plasma samples of individual fish. The lowest cortisol levels occurred in fish caught by seine-netting and killed immediately by anaesthesia in MS 222 (Sandoz). Seine-netting and killing by concussion induced high cortisol levels, in the range of 3-5

{mu}g/100ml. Cortisol levels in fish caught by gill-netting for 18h were 3-6 times higher than in seine-netted fish, and the method of killing was immaterial in this case. The length of time spent in the gill-net had no significant effect on cortisol level, but maintenance of the fish in aquaria for 24-80h elicited high cortisol levels of >50 {mu}g /100ml. Post-mortem delay before blood-sampling resulted in lowered cortisol levels. Seasonal variations in cortisol level were determined in gill-netted fish throughout the year, and in seine-netted fish when available. Cortisol levels were high in both sexes in Sept; and very high in ovulating female caught on the spawning-grounds, though not in ovulating female caught off the spawning-grounds.
Descriptors: reproduction, seasonal variations, blood, spawning, life cycle, *Coregonus lavaretus*, British Isles, Scotland, Lomond L

Goping G, Pollard HB, Adeyemo OM, Kuijpers GAJ (1995) **Effect of MPTP on dopaminergic neurons in the goldfish brain: a light and electron microscope study.** *Brain Research.* 687:35-52
Descriptors: neurobiology, dopaminergic neurons, MPTP, goldfish, CNS, microscopy

Guthrie DM (1983) **Integration and control by the central nervous system**. In: *Control Processes in Fish Physiology* (ed. by J.C. Rankin, T.J. Pitcher & R.T. Duggan), pp. 130-154. Croom Helm, London
Descriptors: neurobiology, physiology, CNS, fish

Hall KC, Bellwood DR (1995) **Histological effects of cyanide, stress and starvation on the intestinal mucosa of *Pomancentrus coelestris*, a marine aquarium fish species.** *Journal of Fish Biology.* 47(3):438-454
NAL Call No. QL614 J68
The histological effects of cyanide, stress and starvation on the gastrointestinal tract of *Pomacentrus coelestris*, a common marine aquarium fish species, were investigated. Neither anaesthetic cyanide nor stress were found to have any detectable effects on the mucosal lining of the intestine. However, starvation resulted in a significant reduction in the intestine length, the surface area of the intestinal mucosa and the mucosal thickness, all occurring within 13 days.
Descriptors: histology, cyanides, digestive system, *Pomacentrus coelestris*, biological stress, aquariology

Hatta K, Korn H (1999) **Tonic inhibition alternates in paired neurons that set direction of fish escape reaction.** *Proceedings of the National Academy of Sciences of the United States of America.* 96:12090-12095
Descriptors: neurobiology, behaviour, fish, tonic inhibition

Hildebrand M (1995) ***Analysis of Vertebrate Structure, Fourth edition.*** John Wiley & Sons, Inc., New York
Descriptors: anatomy, physiology, neurobiology, fish

Hon WK Ng TB (1986) **Hormones with adrenocorticotropic and opiate-like activities from the carp (*Cyprinus carpio*) pituitary**. *Comparative Biochemistry and Physiology.* 85C:443-448
NAL Call No. QP1 C6

Descriptors: physiology, hormones, pituitary, adrenocorticotropic, opiate, carp, *Cyprinus carpio*

Horsberg TE (1994) **Experimental methods for pharmacokinetic studies in salmonids.** *Annual Review of Fish Diseases.* 4:345-358
NAL Call No. SH171 A56
Many aspects of the use of chemical agents to combat diseases in aquaculture should be based on a firm knowledge of their pharmacokinetic behaviour in fish. The environmental conditions (temperature, salinity, pH etc.) under which kinetic studies are conducted, may vary greatly. Pharmacokinetic experiments to determine the rate and magnitude of absorption from water or feed, distribution, qualitative and quantitative metabolism and excretion in fish under various environmental conditions, are important for the determination of correct dosage regimens and withdrawal periods. These studies are often technically very difficult to carry out. Several techniques and experimental designs for different kinetic experiments are described in this review. Techniques requiring considerable manipulation of the fish, such as anaesthesia, catheterisation, cannulation, and immobilisation in metabolism chambers, will subject the fish to significant stress, which in turn may influence the data generated. The parameters reported thus often show considerable divergence. The influence of the experimental design on the results obtained has rarely been studied or addressed in papers describing pharmacokinetic studies in fish. In future studies, more attention should be paid to validation of the experimental methods.
Descriptors: fish culture, fish diseases, disease control, pharmacology, drugs, Salmonidae, environmental factors
ASFA; Copyright © 2003, FAO

Hussain MG (1998) **Manipulation of chromosomes in fish: Review of various techniques and their implications in aquaculture.** *Bangladesh Journal of Fisheries Research.* 2(1):99-108
ISSN: 1026-6690
Human ingenuity has made it possible to advent the chromosome manipulation techniques to produce individuals with differing genomic status in a number of fish using various causal agents such as physical shocks (temperature or hydrostatic pressure), chemical (endomitotics) and anaesthetic treatments either to suppress the second meiotic division shortly after fertilization of eggs or to prevent the first mitotic division shortly prior to mitotic cleavage formation. This results in the induction of polyploidy (triploidy and tetraploidy), gynogenesis (both meiotic and mitotic leading to clonal lines) and androgenesis in fish population. The rationale for the induction of such ploidy in fish has been its potential for generating sterile individuals, rapidly inbred lines and masculinized fish, which could be of benefit to fish farming and aquaculture. In this paper, these are critically reviewed and the implication of recently developed chromosome manipulation techniques to various fin fishes is discussed.
Descriptors: aquaculture techniques, fish culture, biotechnology, reproduction, chromosomes, cell division, fish eggs, polyploids, gynogenesis, androgenesis, clones, hybrid culture
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Jansen GA, Green NM (1970) **Morphine metabolism and morphine tolerance in goldfish**. *Anesthesiology* 32:231-235
Descriptors: neurobiology, physiology, morphine, goldfish

Jerrett AR, Stevens J, Holland AJ (1996) **Tensile properties of white muscle in rested and exhausted chinook salmon (*Oncorhynchus tshawytscha*).** *Journal of Food Science.* 61(3):527-532
NAL Call No. 389.8 F7322
After 40 hr storage at 2°C, the tensile strength of "rested" king salmon (*Oncorhynchus tshawytscha*) "white" muscle was 2.7 times that of the "exhausted" muscle with the "rested" muscle retaining its immediate post-capture strength. A combination of behavioral conditioning, conservative handling practices and chemical anaesthesia (AQUI-S™) was used to minimize the extent of pre-mortem exercise and thereby provide "rested" fish. Postmortem electrical stimulation of the "rested" animals was used to produce "exhausted" muscle. This study highlights the importance of reducing pre-harvest exercise in the production of high quality fish muscle.
Descriptors: muscles, mechanical properties, *Oncorhynchus tshawytscha*, storage effects, human food, fish handling, quality control, tensile strength
ASFA; Copyright © 2003, FAO

Kebus MJ, Collins MT, Brownfield MS, Amundson CH, Kayes TB, Malison JA (1992) **Measurement of resting and stress-elevated serum cortisol in rainbow trout *Oncorhynchus mykiss* in experimental net-pens.** *Journal of the World Aquaculture Society.* 23(1):83-88
NAL Call No. SH138 W62
A commercially available heterogeneous, solid-phase tube enzyme-linked immunoassay (ELISA) was modified and validated for the measurement of serum cortisol in rainbow trout *Oncorhynchus mykiss*. The assay is accurate and precise. Resting and stress-elevated serum cortisol concentrations were measured in rainbow trout with a sensitivity of 1.5 ng/ml. Fish held in net-pens at a density of 0.4 $kg/m^3/cm$ had a resting cortisol level of 16.5 ± 3.8 ng/ml (mean ± SE). At 3 h post-disturbance, serum cortisol levels were not affected by the removal of fish from adjacent net-pens with dip nets or by the use of 200 mg/L tricaine methanesulfonate (MS-222) as an anesthetic for obtaining samples. However, an acute stress (60 s removal from water) elevated serum cortisol levels to 73.7 ± 9.4 ng/ml.
Descriptors: fish culture, cage culture, biological stress, bioassays, serum, *Oncorhynchus mykiss*, ELISA, corticosteroids
ASFA; Copyright © 2003, FAO

Kikuchi T, Sekizawa Y, Ikeda Y, Ozaki H (1974) **Behavioral analyses of the central nervous system depressant activity of 2-ammino-4-phenylthiazole upon fishes.** *Bulletin of the Japanese Society of Scientific Fisheries.* 40(4):325-337
NAL Call No. 414.9 J274
According to the modified McFarland's criterion and the diagrammatical display procedure, behavioural analyses of the central nervous system depressant activity of 2-amino-4-phenylthiazole, a piscine anesthetic, were carried out. Carp (*Cyprinus carpio*) as a representative of a fresh water type, rainbow trout (*Salmo gairdnerii irideus*) as a mid type between fresh and salt water and yellowtail (*Seriola quinqueradiata*) as a salt water type were used. The analyses resulted in establishing a methodological standardization procedure for the application of anesthetics upon a given fish for handling and transportation for aquacultural use. For the bathing anesthetization of carp, a conc of 30-40 ppm provided good anesthesia for 20-40 min. For the bathing sedation, a conc at 12 ppm provided good sedation for 3-72 hr. For the bathing anesthetization of rainbow trout, a conc at 20-30 ppm provided good anesthesia for 40 min to 3 hr and for the bathing sedation, a conc at 10 ppm provided

good sedation for 24 hr. For the bathing anesthetization of yellowtail, a conc at 15-20 ppm provided good anesthesia for 10-25 min. For the bathing sedation, a conc at 8 ppm provided good sedation for 4.5 hr.
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Descriptors: 2-ammino-4-phenylthiazole, CNS, neurobiology, anesthesia, anesthetic, fish

Kotrschal K, van Staaden MJ, Huber R (1998) **Fish brains: evolution and environmental relationships**. *Reviews in Fish Biology and Fisheries*. 8:373-408
Descriptors: neurobiology, CNS, evolution, fish

Le Moal M, Simon H (1991) **Mesocorticolimbic dopaminergic network: functional and regulatory roles.** *Physiological Reviews*. 71:155
NAL Call No. 447.8 P563
Descriptors: neurobiology, mesocorticolimbic dopaminergic network, physiology

Lett BT, Grant VL (1989) **The hedonic effects of amphetamine and pentobarbital in goldfish**. *Pharmacology Biochemistry and Behavior*. 32:355-356
NAL Call No. QP901 P4
Descriptors: biochemistry, goldfish, amphetamine, pentobarbital

Matthews G, Wickelgren WO (1978) **Trigeminal sensory neurons of the sea lamprey.** *Journal of Comparative Physiology* 123:329-333
NAL Call No. 444.8 Z3
Descriptors: physiology, trigeminal, neurobiology, sea lamprey

Mattioli R, Aguilar C, Vasconcelos L (1995) **Reinforcing properties of the neuropeptide substance P in *Carassius auratus:* evidence of dopaminergic system involvement**. *Pharmacology Biochemistry and Behavior* 50:77-81
NAL Call No. QP901 P4
Descriptors: neurobiology, substance P, dopaminergic system, neuropeptide, *Carassius auratus*

Mattioli R, Santangelo EM, Costa ACC, Vasconcelos L (1997) **Substance P facilitates memory in goldfish in an appetitively motivated learning task**. *Behavioural Brain Research*. 85:117-120
Descriptors: goldfish, neurobiology, behaviour, substance P, neuropeptide

Mok EYM, Munro AD (1998) **Effects of dopaminergic drugs on locomotor activity in teleost fish of the genus *Oreochromis* (Cichlidae): involvement of the telencephalon**. *Physiology and Behavior*. 64:227-234
NAL Call No. QP1 P4
Descriptors: telencephalon, physiology, neurobiology, dopaminergic drug, teleost, *Oreochromis*

Mueller R (1976) **Investigations on the body temperature of freshwater fishes.** *Arch feur Fischereiwissenschaft* 27(2):1-28
NAL Call No. SH1 A72
Body temps of brown trout, rainbow trout, perch, pike, chub, barbel and eel have been measured using orally and surgically implanted temp transmitters. Temps of resting fish did

not differ significantly from the temp of the ambient water. Vigorous struggling in a net caused the muscle temp to rise to a maximum of 0.72°C above ambient. During continuous swimming in the fish wheel the body temp did not rise substantially, but after swimming, moderate temp rises were usually observed. Adjustment of body temp after activity to the water temp took place within 20 to 100 minutes. Feeding increased the body temp mainly in conjunction with swimming activity. Time for body temp adaptation in living fish after thermal shock is two thirds of that observed in dead fish, which again depends on a body wt-length relation. Blood circulation intensity is essential for thermal exchange in large fish and can be influenced by an anaesthetic (MS222). Under normal conditions, no specific differences in thermal regime were found among the fish spp tested.
Descriptors: water temperature, temperature effects, methodology, body temperature, thermoregulation, length-weight relationships, blood circulation, controlled conditions, Pisces, *Anguilla anguilla*, Leuciscus, Barbus, *Perca fluviatilis*, *Esox lucius*, *Oncorhynchus mykiss*, *Salmo trutta*
ASFA; Copyright © 2003, FAO

Munro AD (1986) **The effects of apomorphine, *d*-amphetamine and chloropromazine on the aggressiveness of isolated *Aequidens pulcher* (Teleostei, Cichlidae).** *Psychopharmacology* 88:124-128
Descriptors: apomorhpine, d-amphetamine, chloropromazine, psychoparmacology, teleost, *Aequidens pulcher*, neurobiology

Munro AD, Dodd JM (1983) **Forebrain of fishes: neuroendocrine control mechanisms**. In: *Progress in Nonmammalian Brain Research, Volume III* (ed. by G. Nisticò & L. Bolis), pp. 2-78. CRC Press Inc, Florida
Descriptors: neurobiology, CNS, neuroendocrine control, fish

Ng TB, Chan TH (1990) **Adrenocorticotropin-like and opiate-like materials in the brain of the red grouper *Epinephelus akaara* (Teleostei: serranidae).** *Comparative Biochemistry and Physiology*. 95C:159-162
NAL Call No. QP1 C6
Descriptors: neurobiology, adrenocorticotropin-like, opiate-like, CNS, red grouper, teleost, *Epineaphelus akaara*

Pearson MP, Stevens ED (1991) **Size and hematological impact of the splenic erythrocyte reservoir in rainbow trout, *Oncorhynchus mykiss*.** *Fish Physiology and Biochemistry* 9(1):39-50
NAL Call No. QL639.1.F583
Rainbow trout (*Oncorhynchus mykiss*) were sampled individually, at rest, following air exposures of up to 8 min, during recovery from a 5 min air exposure or after a 5 min chase. The spleen was photographed in vivo at rest and following 5 min air exposure in one fish. The effect of individual versus serial sampling from the same tank and of MS222 anaesthesia was also examined. Spleen hemoglobin content (SpHb), spleen somatic index (100 x spleen weight/body weight; SSI), blood hemoglobin concentration (Hb), and hematocrit (Ht), were measured. Mean cell hemoglobin concentration (MCHC), erythrocyte reservoir size, and relative contributions of reservoir release, erythrocyte swelling, and plasma water loss to hemoconcentration were calculated.
Descriptors: erythrocytes, spleen, fish physiology, body weight, *Oncorhynchus mykiss*,

Salmo gairdneri, haemoglobins, somatic index
ASFA; Copyright © 2003, FAO

Piddington RW (1971) **Central control of auditory input in the goldfish. 2. Evidence of action in the free-swimming animal.** *Journal of Experimental Biology.* 55(3):585-610
NAL Call No. 442.8 B77
(1) In the free-swimming electrode-implanted goldfish, the neural response in the medulla to a constant auditory stimulus may exhibit reversible fluctuations in amplitude which are abolished by anaesthesia. (2) The results are consistent with the action of an auditory control system which can reduce or enhance the input following a click. (3) Noise-masking effects and reflex muscular control were excluded by demonstrating the relative constancy of the rectified microphonic during simultaneous changes in the clic-evoked action potential at the medulla. (4) There are 3 kinds of response modification: habituation, rapid inhibitory feedback, and facilitation. (5) Both feedback and habituation act predominantly on high-threshold auditory fibres. Low-threshold fibres do not become habituated, and dishabituation does not occur. (6) As in the mammal, anaesthetic reduces the tendency of the system to become habituated by an amount which depends on the dosage. Auditory fibres with highest threshold have the greatest tendency to become habituated and are the least affected in this respect by anaesthetic. (7) Simple conditioning experiments indicate that control influences exerted over the input can be biased by positive or negative reinforcement which follows the auditory stimulus. (8) The control system may work in attention, in frequency analysis, or in suppressing input to self-made sounds. (9) A new hypothesis is made on the biological significance of hearing in fish. A fish may be able to tell if other swimming fish are approaching, receding, or moving tangentially by analysing the proportions in time of the compressions and rarefactions present in the swimming sounds, which are proposed to be asymmetrical.
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Descriptors: goldfish, audition, hearing, neurobiology, medulla

Saliou A (1980) **Contribution to the study of the inert gas narcosis and high pressure nervous syndrome. Respective effects of the hydrostatic pressure and of the inert hyperbaric pressure.** 103 pp
This thesis studies the hydrostatic pressure effects until 151 atm on the trout (*Salmo trutta*) in experimental conditions. The high pressure effects of several inert gases (nitrogen, helium, argon) are examined by observing the respiratory ventilation, the nervous regulation and the heart rhythm. Some anesthetic substances first at barometric pressure, then at hydrostatic pressure are used to compare their narcotic effects on the fish physiology and behavior. The methodology and experimental conditions are described.
Descriptors: pressure effects, rare gases, helium, nitrogen, argon, nervous system, heart, respiration, physiology, *Salmo trutta*
ASFA; Copyright © 2003, FAO

Sills JB, Allen JL (1971) **The influence of pH on the efficacy and residues of quinaldine.** *Transactions of the American Fisheries Society.* 100(3):544-545
NAL Call No. 414.9 Am3
Quinaldine, an anaesthetic for fish, loses its effectiveness in solutions having pH values< 6. Measured quantities of un-ionized quinaldine in solution compared favourably with calculated values at selected pHs. Quinaldine residues in fish and un-ionized quinaldine in

solution were measured by gas chromatography.
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Descriptors: pH, quinalidine, anesthetic, fish

Snedden LU, Braithwaite VA, Gentle MJ (2003) **Do fish have nociceptors: Evidence for the evolution of a vertebrate sensory system.** *Proceedings of the Royal Society : Biological Sciences.* 270(1520):1115-1121
Descriptors: fish, pain, nociception, trout, teleost, CNS, behaviour, physiology, noxious stimuli
http://gessler.ingentaselect.com/vl=2030559/cl=122/fm=docpdf/nw=1/rpsv/cw/rsl/09628452/v270n1520/s2/p1115

Stobo WT (1972) **Effects of formalin on the length and weight of yellow perch.** *Transactions of the American Fisheries Society.* 101(2):362-364
NAL Call No. 414.9 AM3
Data for fish growth studied often derive from formalin preserved specimens, but little information is available on the effect of the preservation on spiny-rayed fishes. The effect of 10% formalin on length and weight of yellow perch (*Perca flavescens*) was checked during an 18.5 months period. 55 perch were left in anaesthetic solution (0.8% ethylether) until death. Length and weight were recorded prior to death, after 1 hour, then after 1 hour in formalin. Observations were repeated on a geometric time scale for 1 week, weekly for 1 month, then monthly for 7 months and finally 18.5 months after killing. Tabled results show immediate shrinkage in small fish, largely complete in 24 hour but an initial increase in large fish with subsequent shrinkage complete in 5 days. No length corrections are thought necessary for preserved perch. In all perch weight showed an initial rapid increase, which slowed for a short period, then a protracted period of increase followed by a period of decrease (135 to 557 days).
ASFA; Copyright © 2003, FAO
Descriptors: formalin, yellow perch, spiny-ray fishes, preservative, anesthetic

Teo Leng-Hong, Chen Ti-Wen (1993) **A study of metabolic rates of *Poecilia reticulata* Peters under different conditions.** *Aquaculture Fish Management.* 24(1):109-117
NAL Call No. SH1 F8
The aim of this work was to investigate the factors that affect the metabolic rates of guppies, Poecilia reticulata, by measuring the oxygen uptakes of guppies individually or in groups in closed vessels to simulate the actual packaging conditions. Metabolic rates of guppies increased with the increase of temperatures. Anaesthetic, 2-phenoxyethanol, suppressed the oxygen consumption rates. Grouped fish also showed lower metabolic rates than individual fish. Light and starvation did not produced any effect. The pH of the water, ammonium and carbon dioxide concentrations had significant effects on the metabolic rates of guppies.
Descriptors: animal metabolism, controlled conditions, *Poecilia reticulata*, environmental factors, oxygen consumption, group effects, light effects, starvation, pH effects, temperature effects, ornamental fish, fish handling, metabolic rate
ASFA; Copyright © 2003, FAO

Torres P, Tort L, DePauw N, Joyce J (1991) **Effects of stress and metal exposure on blood parameters and liver metabolism in rainbow trout.** *Aquaculture and the Environment, Special Publication, (European Aquaculture Society).* 14: 312-313

NAL Call No. SH138.S64

Amongst the main toxicants for fish, heavy metals have been shown to induce a wide range of effects including changes in respiratory and haematological parameters or energetic resources. At the same time, molecular responses can also be detected such as induction of metallothioneins in liver, low molecular metal-binding proteins involved in heavy metal detoxification. The occurrence of heavy metal contamination can be related as well to an stressing situation, since the consequences of contamination lead to similar changes in some particular physiological indicators (Torres et al. 1986). Moreover it has been shown that both metal treatment and stress would rise the degree of lipid peroxidation due to an increase in free radical production. In this work we try to correlate the fish responses to both metal toxic concentrations and handling stress by analyzing a number of metabolic, physiological or molecular parameters. Thirty-two rainbow trout (*Oncorhynchus mykiss*) obtained from a fish farm were divided in four experimental groups corresponding to control, injected intraperitoneally with saline, injected with cadmium 20 ppm as cadmium chloride and stressed. The stress procedure consisted in handling stress by holding the fishes in the net out of the tank during a period of 10 sec. and repeating this three times a day during one week. After experimental treatments fish were subjected to anaesthesia with phenoxiethanol. Blood (1.5 to 2 ml) was taken off in less than 1 minute. The liver was then excised and the fish weighed and measured. Routine haematological analysis were performed and the concentration of total proteins, glucose and cortisol were determined from plasma. From liver tissue the levels of hepatic protein, metallothionein, thiol groups, peroxides and zinc were determined.

Descriptors: biological stress, pollution effects, heavy metals, water quality, aquaculture facilities, fish culture, hematology, *Oncorhynchus mykiss*

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Tort L, Flos R, Hughes GM (1986) **Methods for studying effects of pollutants on cardiorespiratory physiology in fish.** *Informes Tecnicos. Instituto de Investigaciones Pesqueras., Barcelona.* No. 131. 32 pp

Pollutant substances mobilized from industrial and densely populated areas have been shown to affect the environment, fish being one of the most affected groups. A variety of methods and techniques regarding respiratory, hematological and cardiovascular physiology are reviewed. Other significant aspects such as anaesthesia, anticoagulants and stress are also included.

Descriptors: pollution effects, analytical techniques, respiration, blood circulation, hematology, fish

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Yamamori K, Hanyu I, Hibiya T (1971) **Electrocardiography of the eel by means of underwater electrodes.** *Bulletin of the Japanese Society of Scientific Fisheries.* 37(2)94-97

NAL Call No. 414.9 J274

In the eel, *Anguilla japonica*, which has a heart with a strong electromotive force, the ECGs were found to be recordable by electrodes placed in the water apart from fish's body. An apparatus so devised that an 'underwater electrode' was attached to either end of a plastic cylinder was submerged on the bottom of an aquarium. When the fish slipped into the cylinder, ECGs were easily recorded, being superimposed upon slow undulation of the base line caused by the respiratory movement of the fish QRS complex of the ECG was marked, while P and T waves were identified with difficulty. The amplitude of QRS complex was about 0.5 mV. This method enabled us to observe the heart rate of the eel under least

disturbed state. Ordinary heart rate was not quite regular. Very slight stimulation was enough to bring about cardiac inhibition, which was also accompanied by a considerable reduction in the amplitude of the QRS. After rough treatment, such as deep anaesthesia or exposure to air, the heart rate showed remarkable compensatory increase, reaching a value a few times higher than the normal level. In the American eel, A. rostrata, QRS was approximately 0.02 mV. This indicates that the electromotive force of the heart in this sp is much smaller than in A. japonica.

Descriptors: eel, *Anguilla japonica*, Anguilla, underwater electrodes, ECG, anesthesia, electrocardiography

Web Resources:

Aspects of Animal Welfare and Aquaculture -A Compendium of Selected Literature by Richard D. Moccia and Kristopher P. Chandroo; Aquaculture Centre, University of Guelph, Guelph, Ontario, Canada
http://www.aps.uoguelph.ca/~aquacentre/aec/publications/welfare-bib.html

3.2. CULTURE, FISHERIES, & RESEARCH RELATED TOPICS

3.2.1. ANGLING

Hauber AB, Parsons GR (2000) **The effect of nesting structure specification on welfare estimation in a random utility model of recreation demand: an application to the demand for recreational fishing.** *American journal of agricultural economics.* 82 (3):501-514.
NAL Call No. 280.8 J822
Descriptors: angling, welfare economics, demand, statistical analysis – utility functions, salmon, trout, bass, access

Konigs E (1988) **Tierschutzaspekte im Fischereirecht [Animal welfare aspects of angling.]** *Deutsche Tierarztliche Wochenschrift.* 95(2):58-60 (In German)
NAL Call No. 41.8 D482
Descriptors: animal welfare, fishes, aquatic animals

Leeuw AD de (1996) **Contemplating the interests of fish: the angler's challenge.** *Environmental Ethics* 18(4):373-390. British Columbia Ministry of Environment, Lands, and Parks.
NAL Call No. GF80.E5
Descriptors: game fishes, angling, sportsmen, ethics, hunting, comparisons, recreation, autecology, animal welfare

Neukirch M (1988) **Sportfischerei und Tierschutz [Sport fishing and animal welfare].** *Deutsche Tierarztliche Wochenschrift.* 95(2):63-64 (In German)
NAL Call No. 41.8 D482
Descriptors: animal welfare, fishes, aquatic animals

Schulz U (1995) **Elektrofischerei unter Tierschutzaspekten. [Electrofishing from animal welfare aspects.]** *Deutsche tierarztliche Wochenschrift.* 102(3):125-127. Fischereiforschungsstelle des Landes Baden-Wurttemberg. DTW.
NAL Call No. 41.8 D482
Through improper application of electrofishing techniques fish may be harmed severely. The injuries can range from inner bleeding to broken vertebrae. Due to these possible injuries each electrofishing procedure requires approval of the fisheries authorities. Only persons with a special license are allowed to fish with electricity. The fisheries authorities only approve electrofishing for reasons stated in the fisheries laws. If an application to fish with electricity is approved, the permission is restricted to certain persons, certain equipment units, certain stretches of water and clearly outlined goals. Damages to fish can be reduced to

a minimum by the strict regulations of the fisheries authorities and by the training of the fishermen.
Descriptors: animal welfare, methods, injuries, legislation and jurisprudence, electric stimulation

Zemke P (1994) **Probleme des Tierschutzes in der Angelfischerei. [Animal protection in game fishing.].** *Deutsche Tieraerztliche Wochenschrift.* 101(5):175-177 (In German)
NAL Call No. 41.8 D482
The legislative frame of animal welfare in fishing is built by the German Animal Protection Act. It is completed by different States' legislation on fishing, which essential parts are based on a number of judicial decisions concerning this subject. To be acquainted with the legal directions is of course a necessity for game fishers. Nevertheless, the real attitude in conformity with the rules of animal protection is determined by a comprehensive knowledge of the species fish and its aqueous biotope. In the Federal Republic of Germany an official fishing license is acquired after a special examination, which gives certain guarantee of experience in this field. In practice, however, a lot of deficiencies are observed in handling this vertebrate. As a consequence, we seek a game fisher who is familiar with our native fish fauna and its biotope, who is responsible with the fish creatures and aims at maintaining a healthy and species-rich fish stock in our waters.
Descriptors: fishes, game fishes, fishermen, line fishing, animal welfare, anglerfish, legislation, fishing methods, occupations, saltwater fishes, legal aspects, fishery protection, game, fish, biotopes, licences, animal welfare
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Web Resources:

Animal Welfare Aspects of Recreational Fishing
http://www.ssaa.org.au/fish.html

Welfare of Angled Fish
Pain and Fish Welfare
http://www.vet.ed.ac.uk/animalwelfare/Fish%20pain/welfare.htm#ANGLED

The Welfare of Our Fish
Keith's Angling Pages
http://www.keithshomepage.currantbun.com/welfare.htm

3.2.2. AQUACULTURE

Alestrom P, De la Fuente J (1999) **Genetically modified fish in aquaculture: Technical, environmental and management considerations.** *Biotecnologia Aplicada* (Cuba). 16(2):127-130
ISSN: 0864-4551
Descriptors: aquaculture, genetic engineering, health hazard, animal welfare, ethics, environmental impact assessment, research, international cooperation, nonhuman, review

Annonymous (1991) **The role of the veterinarian in fish farming and aquaculture**. *Veterinary Record* (England). 129(6):124-125.
NAL Call No. 41.8 V641
Descriptors: fisherie, standards, fishes, veterinary medicine, animal welfare, consumer product safety, fish products

Baeverfjord G, Aasgaard T, Lein I, Rye M (1999) ***Egg Incubation Temperature is a Critical Factor for Normal Embryonic Development in Atlantic Salmon.*** International Council for the Exploration of the Sea. Copenhagen (Denmark) Theme Sess. Health and Welfare of Cultivated Aquatic Animals. Council Meeting of the International Council for the Exploration of the Sea, Stockholm (Sweden), 27 Sep-6 Oct 1999. 5 pp. Compact Disc
Farmed fish is marketed as high quality products and as healthy foods. Producers are faced with increasing requirements for documentation on sound farming practices, i.e. contents of fish feeds, drug use etc. The average consumer may perhaps be satisfied to know that the fish itself is healthy and normal at the time of slaughter. Deformities in farmed fish clearly represent a problem in this context. If fish with deformities reach the market, consumers may react adversely. A strategy in which deformed fish is produced and subsequently rejected before marketing is hazardous as well as expensive, and can hardly be sustained for a prolonged period of time. And, although individuals displaying deformities may be regarded as a normal feature of any biological population, sudden increases in number of deformed specimen clearly indicates suboptimal conditions. Therefore, substantial efforts were made by the Norwegian industry, as problems with deformed fish came to attention some years ago. During a period of 4-5 years in the mid-nineties, a variety of malformations were observed in farmed Atlantic salmon (*Salmo salar*) in Norway. The observations caused great concern, both as a cause of considerable economical loss to the fish farmers and as an issue of ethical concern. In a project which was initiated by Norwegian Research Council and supported by industry partners, possible causes for malformations were screened.
Descriptors: embryonic development, temperature effects, fish culture, abnormalities, *Salmo salar*
ASFA; Copyright © 2003, FAO

Baeverfjord G (1998) *Ethics and Animal Welfare in Intensive Aquaculture Production.* Copenhagen Denmark ICES. 3 pp.
Descriptors: aquaculture development

Begout ML, Lagardere JP (1999) ***Effects of Stocking Densities on Swimming Characteristics of Raibow Trout: Applying Acoustic Telemetry to the Culture Environment.*** International

Council for the Exploration of the Sea Copenhagen (Denmark) Theme Sess. Health and Welfare of Cultivated Aquatic Animals. Council Meeting of the International Council for the Exploration of the Sea, Stockholm (Sweden), 27 Sep-6 Oct 1999. 1 pp. Compact Disc
The authors designed an experiment in order to evaluate the effects of stocking densities on behavioural plasticity considered as potential welfare indicator. The authors measured swimming activity of rainbow trout (*Oncorhynchus mykiss*) cultivated at three stocking densities : 25, 75 and 125 kg/m^3. Using acoustic telemetry in tanks, the authors measured swimming activity during 48 h for three fish reared at each density. Water renewal rate was proportionate to fish density to ensure equal water quality and current speed was also similarly regulated. Fish were fed at 1 % ratio through automatic feeders. Strong differences in swimming activity appeared for activity patterns and amplitude. Fish reared at 25 kg/ m^3 showed identical patterns and were day active with a maximum level of 117 m/h. One fish reared at medium density displayed a similar pattern to the 25 kg/m^3 fish, whereas the other two showed high pattern variability and high swimming activity, up to 221 m/h. At the highest density, the three fish showed similar even patterns with a maximum swimming amplitude of 180 m/h. Other swimming Descriptors such as space use, swimming complexity and instantaneous swimming speeds also differed in each density. From this first experiment, we can infer that behavioural plasticity exists at medium density, whereas swimming activity is constrained at the highest density. (DBO).
Descriptors: Stocking density, *Oncorhynchus mykiss*, behaviour
ASFA; Copyright © 2003, FAO

Bell A, Bron J, Turnbull JF, Adams CE, Huntingford FA (2002) **Factors influencing the welfare of farmed Atlantic salmon (*Salmo salar*) in commercial marine cages.** *Research in Veterinary Science* 72(Supplement A):7-8
NAL Call No. 41.8 R312
Descriptors: aquaculture, behavior, philosophy and ethics, *Salmo salar*, Atlantic salmon, animal welfare, behavior, commercial fish farm, marine cage, space utilization, stocking density

Bell JG, McGhee F, Campbell PJ, Sargent JR (2003) **Rapeseed oil as an alternative to marine fish oil in diets of post-smolt Atlantic salmon (*Salmo salar*): changes in flesh fatty acid composition and effectiveness of subsequent fish oil "wash out".** *Aquaculture –* Amsterdam. 218(1-4):515-528
NAL Call No. SH1 A6
Descriptors: nutrition, fish oil alternative, fish physiology, *Salmo salar*, Atlantic salmon

Bernoth EM (1991) **Intensivhaltung von Susswasserfischen. [Intensive culture of fresh water fish.]** *Deutsche tierarztliche Wochenschrift* (Germany). 98(8):312-316. (In German with English summary)
NAL Call No. 41.8 D482
Recently, eel recirculation systems have provoked increasing attention in intensive fish culture, especially concerning animal welfare. "Aquaculture", "Intensive Culture", and "Recirculation Systems" are often confused with each other. This study, first of all, differentiates among these terms. The economic relevance of aquaculture in the Federal Republic of Germany is demonstrated by figures. A tendency towards intensive methods can only be seen in trout and eel culture. The problems of recirculation systems are explained comprehensively. Particular emphasis is laid on the conflict between theoretical suitability of intensive fish production and the absence of commercially working systems. At present,

intensive fish culture does not pose a serious animal welfare problem in Germany. However, it is necessary to define the biological requirements of fish concerning their optimal accommodation in aquaculture facilities. According to the Law for the Protection of Animals, these requirements have to be laid down in an ordinance. Only then control is possible as to whether aquaculture systems fulfill fish welfare demands.
Descriptors: animal husbandry, methods, animal welfare, legislation and jurisprudence, fisheries, fishes, growth and development, Germany

Brandt TM, Graves KG, Berkhouse CS, Simon TP, Whiteside BG (1993) **Laboratory spawning and rearing of the endangered fountain darter.** *Progressive Fish Culturist*. 55(3):149-156
NAL Call No. 157.5 P94
Survival of the fountain darter (*Etheostoma fonticola*), a U.S. federally listed endangered species, may depend on captive propagation. Studies were conducted to determine the effect of temperature on spawning and to develop methods for culture. The fountain darter spawned and produced viable offspring in aquaria at 27, 24, 21, 18, 15, 12, 9, and 6°C. The fish also spawned at 3 and 30°C but did not produce viable eggs. Daily egg production of individual fish held at 27, 21, 15, and 9°C was variable. The mean critical thermal maximum for the fountain darter was 34.8°C. Early life stages, 4-14 mm long, were offered a variety of live protozoans, rotifers, and microcrustaceans. Food selection varied with fish size and food size. Fountain darters reached sexual maturity in about 180 d when maintained at 21°C. Three-year-old darters produced viable offspring, and several lived longer than 4 years. Tricaine methanesulfonate was an effective anesthetic at 60 mg/L but was fatal to subadults at 100 mg/L.
Descriptors: rare species, fish culture, *Etheostoma fonticola*, water temperature, spawning, food organisms, USA, Texas, San Marcos R., laboratory culture, aquaculture
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Broom DM (1998) **Fish welfare and the public perception of farmed fish.** (Eds:) Nash CE, Julien V. *Report Aquavision '98 The second Nutreco Aquaculture Business Conference Stavanger Forum, Norway, 13 15 May 1998 Addressing the challenges to maintain a sustainable 10 20% annual growth of the aquaculture industry.* Stavanger Norway Nutreco Aquaculture. pp. 89-91
A discussion is presented on fish welfare and the fish farming industry. Recommendations for improving fish welfare are provided under the following headings: 1) stocking density; 2) feeding methods; 3) stunning; 4) environmental quality enrichment; 5) disease and parasitism; and, 6) handling, grading and transport. It is concluded that the fish farming industry has to have a good image with the public in relation to animal welfare. The industry is vulnerable at the moment, however with some relatively inexpensive changes, the welfare of farmed fish can be good and this fact may be used in marketing.
Descriptors: fish culture, fish diseases, husbandry diseases, environmental diseases, food fish, marketing
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Brown L (1991) **Farmed fish**. Anderson, R.S.; Edney, A.T.B. (eds.) *Practical animal handling.* Pergamon Press plc, Oxford, UK. p. 167-169
NAL Call No. SF61 P73 1991
Descriptors: fishes, fish diseases, transport of animals, animal welfare, restraint of animals, fish culture, animal diseases, animal health, animal husbandry, methods, aquaculture, transport

Chatain B, Corrao D (1992) **A sorting method for eliminating fish larvae without functional swimbladders.** *Aquaculture.* 107(1):81-88
NAL Call No. SH1 A6
The authors describe a simple sorting method for separating cultured fish larvae with functional swimbladders from those without based on density differences. The whole population was first anaesthetized with MS 222 and then the fish were separated: fish with a functional swimbladder float and those without sink. The efficiency of the separation method was tested at several anaesthetic doses (0.02 to 0.1 g/l) with sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus auratus*) larvae in the 6-34 mm (total length) range. The minimal sorting size was 15 mm for sea bass with an optimal anaesthetic dose of 0.07 g MS 222/l. There were not enough data to draw conclusions for sea bream. The method was satisfactory when applied in real conditions to a large (90,000) population of sea bass fry with an efficiency ratio of over 80%.
Descriptors: fish larvae, aquaculture techniques, fish culture, *Dicentrarchus labrax*, *Sparus aurata*, swim bladder, separation processes
ASFA; Copyright © 2003, FAO

Cooke M (2001) **Ethical considerations for the production of farmed fish--the retailer's viewpoint.** (Eds:) Kestin SC, Warriss PD. *Farmed Fish Quality.* Osney Mead Oxford OX2 0EL UK. Blackwell Science Ltd. pp. 116-119
NAL Call No. SH151 F37 2001
It may appear anomalous in a chapter about ethics in the context of fish quality, but I am focusing here on trade. Ethics, even in this context, is not just a synonym for animal welfare; it is the cornerstone of exchange. It can be argued that the production and marketing of farmed fish is the subject of four ethical domains: (1) Employment (2) Trading (3) Animal husbandry, and (4) Environment.
Descriptors: fish culture, seafood , quality control, sociological aspects
ASFA; Copyright © 2003, FAO

Costello MJ, Grant A, Davies IM, Cecchini S, Papoutsoglou S, Quigley D, Saroglia M. (2001) **The control of chemicals used in aquaculture in Europe.** *Journal of Applied Ichthyology.* 17(4):173-180
NAL Call No. QL614 Z44
A range of chemicals are used in European marine aquaculture and these may be categorized as disinfectants, antifoulants and medicines (includes vaccines). This article provides a review of chemicals used in aquaculture in Europe, their regulatory status, and a checklist of points considered best practice in the use (and avoidance of use) of medicines in marine aquaculture. The release of antifoulants and disinfectants into the marine environment is controlled by local and/or national waste discharge regulations that may in turn be guided by wider environmental quality objectives. The authorization of veterinary medicines, biologicals (vaccines) and pharmaceuticals (chemicals), in Europe is the subject of several EC Directives. Registration dossiers address the issues of product quality, safety and efficacy and include environmental and consumer safety where the product is destined for use in a food-producing animal. Fish farmers, like all livestock producers, must have access to a range of properly authorized medicines to safeguard animal health and welfare. The distribution and supply of medicines must be appropriately controlled and their authorization appropriately includes environmental risk assessment to a common European Union (EU) or international standard. There is progress towards the harmonization of the authorization

process within the EU and this will help to ensure the continued availability of medicines for fish. Consumer safety is addressed by the setting of maximum residue limits (MRLs) derived through toxicological risk assessment and by surveillance of food for residues of veterinary medicines. The system for the environmental risk assessment of chemicals used in aquaculture is being developed and is outlined in the present article. It is recommended that the supply and use of fish medicines is uniformly regulated in the EU and supported by appropriate codes of best practice. A number of codes of practice that include reference to the use of medicines have been produced both at a European level and in member states. It is recommended that all European marine aquaculture producers adopt a code of best practice for the use of medicinal and other chemicals their industry. Medicines are one part of an integrated package in dealing with animal health. This includes environmental conditions, nutrition and hygiene. The best practice guidelines presented here are based on the outcome of three European workshops as part of the EU MARAQUA project that involved industry, government and research scientists. They cover the avoidance and minimizing of the need to use medicines and other chemicals, to recording and monitoring their use and effectiveness (in case of resistance development), exchange of experiences within the industry, and staff training. Recommendations are also included for manufacturers of medicines and other chemicals, and for regulatory authorities. Minimizing the need to use medicines and other chemicals requires attention to a healthy source of fish stock. Staff must be appropriately trained in fish husbandry (to minimize stress), hygiene and disease recognition and treatment, including management of the farm site to keep it disease free. The latter may require single generations of fish per site to allow a fallow period during which a disease or parasite cycle is broken. These recommendations and guidelines are in accordance with the current codes of practice being developed by different sectors of the aquaculture industry in different countries. They do not necessarily involve significantly higher production costs and indeed are more likely to save costs as medicines and disease impacts are very costly to industry.
Descriptors: marine aquaculture, medicine, disinfectants, antifouling substances, waste disposal, legal aspects, *Salmo salar, Oncorhynchus mykiss, Dicentrarchus labrax, Sparus aurata*

Dobosz S, Kuzminski H (1997) **Full cycle production of the Pomeranian Gulf whitefish.** *Polskie Archiwum Hydrobiologii / Polish Archives of Hydrobiology.* 44(1-2):287-292

In the Inland Fisheries Institute, Salmonid Research Laboratory Rutki the successful attempt of the Pomeranian Gulf whitefish (*Coregonus lavaretus*) full cycle production was performed. In November 1994 the 154 farmed whitefish females were spawned and 16 liters of fertilized eggs obtained. Before spawning fish were immobilized with "Propiscin" anesthetic, which prevented post spawning broodfish mortality. The hatching was delayed by the incubation in controlled (low) temperature, to obtain alevins during favourable water temperatures. During the first 3 weeks fry were fed with brine shrimps exclusively until obtaining 23.5 mg mean body weight. During the next 4 weeks fish were fed with brine shrimps and trout starter feed alternatively, until fry obtained individual weight 163 mg. During the next 5 weeks fish were fed with trout feed exclusively until the weight 0.95 g. The survival from hatch being 72%. It was proved that the "trout" technology is usefull in whitefish fingerlings and elder fish production.
Descriptors: fish culture, life cycle, induced breeding, mortality, hatching, brood stocks, aquaculture techniques, developmental stages, temperature preferences, *Coregonus lavaretus*, Poland, Baltic whitefish

Eknath AE, Dey MM, Rye M, Gjerde B, Abella TA, Sevilleja R, Tayamen MM, Reyes RA, Bentsen HB (1998) **Selective breeding of Nile tilapia from Asia.** *Proceedings of the 6th World Congress on Genetics Applied to Livestock Production, Armidale, Australia, January 11-16, 1998. Volume 27: Reproduction; fish breeding; genetics and the environment; genetics in agricultural systems; disease resistance; animal welfare; computing and information technology; tree breeding.* World Congress on Genetics Applied to Livestock Production. Armidale, Australia. 89-96

NAL Call No. SF105 W67 1998

A programme implemented by International Center for Living Aquatic Resources Management and other research institutes for the genetic improvement of growth rate in Nile tilapia (*Oreochromis niloticus*) is described. It involves (1) the assembling of tilapia germplasm from wild habitats in Africa and from domesticated Asian aquaculture stocks, (2) estimation of genotype x environment interaction in 11 test environments covering diverse Asian farming systems, (3) a complete diallel crossing experiment involving 23 779 individuals in crossbred and pure strain combination to estimate heterosis, and (4) selection for growth rate. Genotype x environment interaction was very low. Heterosis averaged 4.3%, the largest value being 14%. In the base population, made up of individuals from the 25 best-performing purebred and crossbred groups, the estimates of heritability for body weight from the sire and dam components of variance were 0.23 and 0.53 respectively. The genetic gain in growth rate per generation over 5 generations of selection was 12-17%. The production potential of the new strain was compared with existing strains in station and farm experiments in 5 Asian countries. It was found that the cost of production per unit of fish was 20-30% lower for the new strain than for other Nile tilapia strains in current use.

Descriptors: body weight, genetic improvement, tropics, tilapia, *Oreochromis niloticus,* fishes

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Ellis T, North B, Scott AP, Bromage NR, Porter M, Gadd D (2002) **The relationships between stocking density and welfare in farmed rainbow trout.** *Journal of Fish Biology.* 61(3):493-531

NAL Call No. QL614 J68

There is increasing public, governmental and commercial interest in the welfare of intensively farmed fish in UK, and stocking density has been highlighted as an area of particular concern. Here, we draw scientific attention and debate to this emerging research field by reviewing the evidence for effects of density on rainbow trout. Although no explicit reference to 'welfare' has been made, there are 43 studies which have examined the effects of density on production and physiological parameters of rainbow trout. Increasing stocking density does not appear to cause prolonged crowding stress in rainbow trout. However, commonly reported effects of increasing density are reductions in food conversion efficiency, nutritional condition and growth, and an increase in fin erosion. Such changes are indicative of a reduced welfare status; although, the magnitude of the effects has tended to be dependent upon study-specific conditions. Systematic observations on large scale commercial farms are therefore required, rather than extrapolation of these mainly small-scale experimental findings. There is dispute as to the cause of the observed effects of increasing density, with water quality deterioration and/or an increase in aggressive behaviour being variously proposed. Both causes can theoretically generate the observed effects of increasing density, and the relative contribution of the two causes may depend upon the specific conditions. However, documentation of the relationship between density and the effects of aggressive behaviour at relevant commercial densities is lacking. Consequently only inferential evidence

exists that aggressive behaviour generates the observed effects of increasing density, whereas there is direct experimental evidence that water quality degradation is responsible. Nevertheless, there are contradictory recommendations in the literature for key water quality parameters to ensure adequate welfare status. The potential for welfare to be detrimentally affected by non-aggressive behavioural interactions (abrasion, collision, obstruction) and low densities (due to excessive aggressive behaviour and a poor feeding response) have been largely overlooked. Legislation directly limiting stocking density is likely to be unworkable, and a more practical option might be to prescribe acceptable levels of water quality, health, nutritional condition and behavioural indicators.
Descriptors: aggressive behaviour, animal behaviour, animal welfare, fish culture, fish farms, overcrowding, stocking density, stress, water quality, rainbow trout, *Salmo*, aquatic

Etscheidt J, Manz D (1992) **Susswasseraquaristik und tierarztliche Praxis. Teil 2: Untersuchungen zur artgerechten Haltung von Zierfischen. [Fresh water aquaria and veterinary practice. 2. Studies of proper raising of ornamental fish.]** *Tierarztliche Praxis* (Germany). 20(2):221-226. (In German with English summary)
NAL Call No. SF603.V4
The environmental conditions in 103 fresh-water aquaria were examined and the results compared to the standards as described in part 1 of this publication. The aim was to assess whether the care of aquarium fish was adequate in regard to management and the legal requirements of animal welfare. Disclosed faults are discussed and recommendations for their elimination and avoidance are suggested.
Descriptors: animal welfare, fishes, physiology, fresh water, chemistry, algae growth and development, animal fee, legislation and jurisprudence, Germany, temperature, microbiology

Ewbank R, Kim-Madslien F, Hart CB (1999) ***Management and Welfare of Farm Animals: UFAW Farm Handbook.*** Universities Federation for Animal Welfare UK, 308 pp.
NAL Call No. SF61 M35 1999
At a time when the quality of management of farm livestock is under threat from economic forces, this new edition is as relevant as ever in pointing the way to optimum conditions. There are chapters on the care of dairy cattle, beef cattle (and veal calves), sheep, goats, pigs, rabbits, red deer, laying hens, broiler chickens, turkeys, ducks, quail production, guineafowl and fish farming. Students of agriculture and of veterinary medicine will benefit from it. Previous editions appeared in 1971, 1978 and 1988.
Descriptors: animal husbandry, animal welfare, livestock, animal production, poultry, nutrition, animal breeding, fishes, cattle, sheep, goats, pigs, rabbits, red deer, aquatic animals

Gregory NG (2000) **Animal welfare in the fish industry.** *Surveillance (Wellington).* 27(2):8-10
NAL Call No. SF604.63.N45S87
Descriptors: animal welfare, fisheries, aquaculture, stress, fishing, fish farming, fishes, aquatic animals

Hussain MG (1998) **Manipulation of chromosomes in fish: Review of various techniques and their implications in aquaculture.** *Bangladesh Journal of Fisheries Research.* 2(1):99-108.
ISSN: 1026-6690
Human ingenuity has made it possible to advent the chromosome manipulation techniques to produce individuals with differing genomic status in a number of fish using various causal

agents such as physical shocks (temperature or hydrostatic pressure), chemical (endomitotics) and anaesthetic treatments either to suppress the second meiotic division shortly after fertilization of eggs or to prevent the first mitotic division shortly prior to mitotic cleavage formation. This results in the induction of polyploidy (triploidy and tetraploidy), gynogenesis (both meiotic and mitotic leading to clonal lines) and androgenesis in fish population. The rationale for the induction of such ploidy in fish has been its potential for generating sterile individuals, rapidly inbred lines and masculinized fish, which could be of benefit to fish farming and aquaculture. In this paper, these are critically reviewed and the implication of recently developed chromosome manipulation techniques to various fin fishes is discussed.
Descriptors: aquaculture techniques, fish culture, biotechnology, reproduction, chromosomes, cell division, fish eggs, polyploids, gynogenesis, androgenesis, clones, hybrid culture
ASFA; Copyright © 2003, FAO

Hurnik JF, Lehman H (1988) **Ethics and farm animal welfare**. *Journal of Agricultural Ethics*. 1(4):305-318.
NAL Call No. BJ52.5 J68
Descriptors: *Salmo gairdneri*, mucus, toxicity, water, pollution, acids, aluminium, zinc, animal morphology, body fluids, bony, fishes, elements, heavy metals, metallic elements, salmonoidei

Kolle P, Hoffmann R (1997) **Qualzuchten bei Fischen [Excessive breeding in ornamental fish].** *Deutsche Tierarztliche Wochenschrift*. 104(2):74-75 (In German with English summary)
NAL Call No. 41.8 D482
Descriptors: breeding, fish, ornamental fishes, aquarium fishes, animal welfare, goldfish, *Carassius*, *poeciliidae*

Lines JA, Frost AR (1999) **Review of opportunities for low stress and selective control of fish.** *Aquacultural Engineering*. 20 (4):211-230.
NAL Call No. SH1.A66
Descriptors: fishes, animal welfare, fish culture, fish farms, equipment, selection, sampling, senses, trauma, diseases, inspection, evaluation, literature reviews

Lefrancois C, Mercier C, Claireaux G (1999) ***Effect of Rearing Density on the Routine Metabolic Expenditure of Farmed Rainbow Trout (Oncorhynchus mykiss).*** Copenhagen Denmark ICES. Council Meeting of the International Council for the Exploration of the Sea, Stockholm (Sweden), 27 Sep-6 Oct 1999. 16 pp. Compact Disc.
For an intensive rearing system, increasing the fish stocking density is one way to optimize productivity. However, a too high density can become stressful for the fish and constrain its growth capacity. This negative effect seems to partly mediate through fish behavioral changes (increase of social interactions: aggression, chase, hampering,..) which induce supplementary metabolic expenditure to the detriment of the fish growth. The objectives of this study are (i) to examine the effects of rearing density on the rainbow trout (*Onchorhynchus mykiss*) routine metabolic rate (RMR), (ii) to estimate the species metabolic scope, which represents the energy available to ensure the animal activities (iii) to determine the share of metabolic scope consumed by the density dependent RMR changes. Respirometry experiments were conducted at three densities: 25, 65 and 100 kg/m^3 with 11°C-acclimated and starved rainbow trout (261 ± 5 g). The only significant effect of density

was shown between 65 and 100 kg /m^3 (100.76 ± 1.28 and 104.72 ± 1.29 mgO_2/kg/h, respectively). No significant difference was observed neither between 25 (102.83 ± 1.47 mgO_2/kg/h) and 65, nor between 25 and 100 kg/m^3. Furthermore, the metabolic scope of the rainbow trout was estimated to 285 mgO_2/kg/h. When analyzed with regard to fish metabolic capacities, the RMR corresponded to less than 16% of the animal scope, and RMR variations to less than 1.4%. In the range tested, the density does not affect the routine metabolism of rainbow trout and is not harmful to the realization of the natural fish activities, such as growth. However, if a density of 100 kg /m^3seems to be applied in routine conditions without increasing oxygen demand, similar studies need to be conducted in different experimental stimulation, e.g. during feeding phase.
Descriptors: rearing, stocking density, animal metabolism, biological stress, aggressive behaviour, *Oncorhynchus mykiss*

Lymbery P (1992) **Welfare of farmed fish.** *Veterinary Record* (England). 131(1):19-20.
NAL Call No. 41.8 V641
Descriptors: animal welfare, fisherie, standard, fishes, Great Britain

Maule AG, Schrock RM, Fitzpatrick MS, Schreck CB (1994) **Immune-endocrine interactions during final maturation and senescence of spring chinook salmon.** *High Performance Fish: Proceedings of an International Fish Physiology Symposium at the University of British Columbia in Vancouver, Canada, July 16-21 1994.* Fish Physiology Association, Vancouver, BC (Canada). pp. 170-171
NAL Call No. QL639.1 I58 1994
Adult Pacific salmon (*Oncorhynchus spp.*) present a unique model for immune-endocrine interactions because the processes of sexual maturation and senescence occur simultaneously. We examined: (1) the ability of peripheral blood leukocytes (PBLs) to generate specific antibody-producing cells (APC) and (2) lysozyme activity in skin, nares, mouth, intestinal mucus, and in serum from the primary and secondary circulation in adult spring chinook salmon (*O. tshawytscha*). We also measured concentrations of steroid hormones in primary and secondary circulation and cortisol receptors in PBLs. Plasma concentrations of the stress hormone cortisol were high (> 200 ng/ml) compared to that of unstressed juvenile salmon (Maule et al. 1989); cortisol decreased (< 175 ng/ml) in fish held under constant environmental conditions. However, these values may not reflect true resting levels because of stress associated with collecting fish and the sublethal doses of anesthetic used (Barton et al., 1986). APCs were low (less than or equal to 250 APC per culture) in fish sampled during their migration, but increased significantly (greater than or equal to 400 APC per culture) when fish were held in constant environmental conditions. While the APC response was not sexually dimorphic, concentrations of several sex steroids were correlated with APC in females but not males. Lysozyme activity was significantly higher in mucus from skin, nares and vent than in serum from primary or secondary circulation.
Descriptors: sexual maturation, biological aging, endocrinology, sex hormones, immunity, spring, *Oncorhynchus tshawytscha*

McLay HA, Youngson AF, Wright RS, Johnstone R (1992) **Effects of rearing density on sexual maturation and growth in sea-cage reared Atlantic salmon, *Salmo salar* L.** *Aquacult. Fish. Manage.* 23(3):353-365.
Paired subgroups of fish were derived in January from each of two parent sea-cages of

Atlantic salmon, *Salmo salar*. The proportion of fish which later became sexually mature in each parent group, after one winter (as grilse) and under commercial rearing density, was determined. Maturity rates in the subgroups, reared in sea-cages at low density, were significantly greater than in the parent groups. Rearing at reduced density was associated with increased growth in some, but not all, comparisons. Periodic anaesthesia, handling and sampling of blood for steroid hormone determinations did not consistently affect maturation rate or growth among fish in one of each pair of subgroups. Sexual development was assessed by determining levels of the steroid hormones 11-oxotestosterone or 17 beta - oestradiol in samples of blood serum taken monthly from individually marked fish in one of each pair of subgroups.

Descriptors: growth, population density, fish culture, sex hormones, *Salmo salar*, density dependence, sexual maturity

Midtlyng PJ, Bjerkas E, Waagbo R, Rodger H, Wall T, Palmer R, Breipohl W (1998) **Cataracts in farmed fish---A multidisciplinary initiative for scientific programme.** *Third european marine science and technology conference MAST conference, Lisbon, 23 27 May 1998: Project synopses Vol 6: Fisheries and Aquaculture FAIR: 1994 98, selected projects from the research programme for Agriculture and Fisheries including agro industry, food technology, forestry, aquaculture and rural development FAIR. Luxembourg Luxembourg European Commission DG 12 Science, Research and Development.* 6:341-343

Cataracts (reversible or irreversible lens opacities) is a production disorder of several species of fish. The aim of this project is to disseminate knowledge on occurrence and causation of cataracts in farmed fish, and to initiate further research to prevent and control the disease. To achieve this goal, we propose to create a multidisciplinary network of European scientists and aquaculture industrialists to overcome present restraints in research. The project will thus contribute to secure the health and well being of farined fish, and improve the cost effectiveness of European aquaculture. The competitive advantage of European suppliers of materials or services to the international aquaculture market will be increased. Successful control of cataracts is considered even more important in order not to compromise consumer perception of the aquaculture industry as such, particular regarding its ethical standards of production and the quality of its products. These issues are of major importance for the industry's further competitiveness in the international food market. Bringing the aquaculture industry, fish pathologies and nutritionists together with multidisciplinary working ophthalmologists, a new and unique scientific collaboration for the benefit of future aquaculture production, fish welfare and fish health research will be created, in itself a most valuable goal. These main objectives have been detailed as follows: The project will disseminate scientific knowledge and state of the art in cataract research, and discuss current and planned work conducted in this field. The project will facilitate exchange of study specimen and transfer of research methodology between laboratories. The project will initiate epidemiological studies and thus provide new scientific data on the occurrence and economy of the disease. New research on the physiology of the fish eye and the pathogenesis of lens cataracts will be stimulated. Financial support for explanatory studies on the cataract problem will be sought.

Descriptors: animal physiology, aquaculture, fish culture, pathology, eyes

Olesen I, Bentsen HB (1999) **Breeding programs for sustainable fish production.** *Nordisk Jordbrugsforskning.* 81(3):258-266

NAL Call No. 11 N752

The definition of breeding goals for sustainable fish production are considered, with

emphasis on ethical as well as economic values. Compared with livestock, fish farming is at an early stage of domestication and breeding, although rapid selection responses for growth rate have already been established in several species. More basic knowledge is needed so that welfare standards and normal behaviour of fish can be maintained during selection programmes. The genetic requirements for a long-term selection programme are discussed including continuous (re)introduction of genetic variability from outside the breeding nucleus without adverse performance consequences.
Descriptors: fish production, aquaculture, breeding programmes, domestication, ethics, fitness, genetic drift, genetic variation, growth rate, inbreeding, natural selection, selection, selection responses, adverse effects, sustainability

Pante MJR, Gjerde B, McMillan I (1998) **Inbreeding levels and inbreeding depression in a farmed population of rainbow trout (*Oncorhynchus mykiss*).** *Proceedings of the 6th World Congress on Genetics Applied to Livestock Production, Armidale, Australia, January 11-16, 1998. Volume 27: Reproduction; fish breeding; genetics and the environment; genetics in agricultural systems; disease resistance; animal welfare; computing and information technology; tree breeding.* World Congress on Genetics Applied to Livestock Production. Armidale, Australia. 119-122
NAL Call No. SF105 W67 1998
Data on a population of rainbow trout that had been selected (mainly for growth rate) for 6 generations were analysed. The level of inbreeding in generations 2-6 averaged 2.9, 2.8, 8.5, 8.0 and 6.6% respectively; corresponding values of the percentage of individuals with inbreeding greater than zero were 59.3, 49.8, 87.1, 82.8 and 81.4. The rate of increase in inbreeding over generations was non-linear, the average being 1.3% per generation. Body weight at harvest was depressed by 0.2% per 1% increase in inbreeding.
Descriptors: inbreeding depression, inbreeding, body weight, rainbow trout, fishes, *Salmo*

Peters G (1990) **Tierschutzprobleme in der Massenhaelterung von Nutzfischen. [Problems concerning animal protection laws in connection with mass culture of fishes.]** *Deutsche Tieraerztliche Wochenschrift.* 97(3):157-160.
NAL Call No. 41.8 D482
Descriptors: fishes, animal husbandry methods, aquaria, animal welfare, large scale husbandry, animal collections, animal health, animal husbandry methods, animals, aquatic animals, aquatic organisms, collections, methods, vertebrates, zootechny

Pironet FN, Jones JB (2000) **Treatments for ectoparasites and diseases in captive Western Australian dhufish.** *Aquaculture International.* 8(4):349-361
NAL Call No. SH1.A627
The Western Australian dhufish (*Glaucosoma hebraicum*), an open-water marine finfish, has been identified as a potential species for aquaculture and a 4 y research project has concentrated on broodstock collection and maintenance, spawning and larval rearing. This paper describes treatments which were developed for the ectoparasites and diseases of broodstock fish. These included bacterial and fungal infections, *Cryptocaryon irritans*, *Haliotrema* sp., an unidentified axinid monogenean, the isopod *Aega cyclops* and copepod *Caligus* sp. Treatments based on betadine, formalin, freshwater, malachite green, oxytetracycline (terramycin), 2-phenoxyethanol, potassium permanganate and trichlorphon (neguvon) were all tried. The most effective treatments against parasites were a freshwater

bath or a combined freshwater bath and anaesthesia with 2-phenoxyethanol. Monogeneans on the gills were difficult to control and exophthalmia was an unresolved problem.
Descriptors: ectoparasites, parasite control, therapy, fish diseases, pathogenic bacteria, aquaculture development, disease control, fungal diseases, *Haliotrema*, *Cryptocaryon irritans*, *Caligus*, *Aega*, *Glaucosoma hebraicum*, ISW, Australia, Western Australia
ASFA; Copyright © 2003, FAO

Poppe TT, Barnes AC, Midtlyng PJ (2002) **Welfare and ethics in fish farming.** *Bulletin of the European Association of Fish Pathologists*. 22 (2):148-151
ISSN: 0108-0288
Descriptors: animal health, animal welfare, fish farming, Atlantic salmon, fishes, *Salmo*, aquatic animals

Roberts R (1979) **The fish farming industry**. *The welfare of the food animals. Proceedings of a UFAW symposium held on 28th-29th September 1978*. Universities Federation for Animal Welfare, 8 Hamilton Close. South Mimms, Hertfordshire, UK. p.64-66
NAL Call No. HV4704 U5
Descriptors: slaughter, carbon dioxide, animal welfare, fish farming, fishes, trout, Salmonidae, Salmoniformes

Schnick RA (1996) **Cooperative Fish Therapeutic Funding Initiative: States in partnership with federal agencies to ensure the future of public fish culture.** *Transactions of the North American Wildlife and Natural Resources Conference.* pp. 553-557
NAL Call No. 412.9 N814
The impetus for the Cooperative Fish Therapeutic Funding Initiative was and is the lack of properly approved drugs to reduce disease-related mortality and improve production efficiency and product quality on public aquaculture facilities. This crisis requires more cost-effective methods to gain approval of drugs for use in public aquaculture. Public concerns about human food safety, human health and environmental impacts have resulted in increasingly strict interpretation and enforcement of regulations by the U.S. Food and Drug Administration (FDA). Such actions have drastically curtailed the availability and use of drugs essential to maintain fish health in hatcheries. Drug and chemical manufacturers are reluctant to undertake any significant efforts to gain approval of aquaculture drugs because the market potential for these products is below the potential sales target for research investment (estimated to be $3.5 million for one fish species and one disease). The approval of a drug by FDA can only be obtained with the development of required safety and efficacy data that leads to a new animal drug application (NADA) that is submitted to FDA for review and approval. The process to generate all the data and have the NADA approved by FDA may take 5 to 10 years. Only three therapeutants and one anesthetic are currently approved and available to hatchery managers. It became apparent to a number of individuals, agencies and organizations that a massive, coordinated and cooperative effort was needed to resolve this crisis. This is the story of how various groups have joined together to meet this awesome responsibility.
Descriptors: drugs, disease control, fish culture, government policy, public health, product development, USA
ASFA; Copyright © 2003, FAO

Schnick RA, Gingerich WH, Koltes KH (1996) **Federal-state aquaculture drug registration partnership: A success story in the making.** *Fisheries.* 21(5):4
NAL Call No. SH1.F54

During the past 20 years, aquaculture has grown both as a vital tool for fisheries management and as a viable industry. But now a crisis has arisen from the Food and Drug Administration's (FDA) increased regulation of drug use in aquaculture in response to public concerns about human food safety, human health, and environmental effects. Lack of approved drugs and chemicals has dramatically reduced the effectiveness and increased the cost of fish production for natural resource management agencies. To make badly needed therapeutants available, the FDA is requiring an array of specialized laboratory research studies and clinical field trials. Pharmaceutical manufacturers are reluctant to undertake any major efforts to gain approval of aquaculture drugs because each (i.e., use on one species for one purpose) is estimated to cost a minimum of $3.5 million. Hence, the expenditure is not warranted by the apparent market potential. Only three therapeutants and one anesthetic are currently approved and available to hatchery managers.
Descriptors: aquaculture products, aquatic drugs, pharmacology, legislation, USA, disease control, aquaculture, pharmaceuticals
ASFA; Copyright © 2003, FAO

Schwedler TE, Johnson SK (2000) **Animal welfare issues. Responsible care and health maintenance of fish in commercial aquaculture**. *Animal Welfare Information Center Bulletin.* 10(3/4):3-9
NAL Call No. aHV4701 A952
Descriptors: aquaculture, animal welfare, angling, fish farming, stress, water quality, stocking density, fish diseases, disease prevention, fishes

Sievers G, Lobos C, Inostroza R, Ernst S (1996) **The effect of the isopod parasite *Ceratothoa gaudichaudii* on the body weight of farmed *Salmo salar* in southern Chile.** *Aquaculture.* 143 (1):1-6
NAL Call No. SH1 A6
A study of the isopod *Ceratothoa gaudichaudii*, a parasite on farmed Atlantic salmon (*Salmo salar*) was carried out on Guar Island, Chile, between May 1993 and August 1994. A total of 671 salmon, with an initial body weight of 0.9 kg to 1.1 kg, was selected; one third were naturally infected with one or two growing parasites. The fish were kept in a separate cage and were examined individually, under anaesthesia, five times during the study. On each occasion, the weight and number of the parasites on each fish was recorded. There was an increase in the prevalence of the parasitosis from 33.4% to 98.2%; concurrently, the total number of parasites on salmon rose from 309 to 3987 with an increase of infestation intensity from 1.4 to 6.1 parasites per fish. No adult females with eggs or larvae were found. At the end of the study, salmon with less than three parasites weighed 4428 ± 949 g; those with three to eight parasites weighed 4151 ± 983 g, and those with more than eight parasites weighed 3763 ± 1056 g. A significant difference in weight ($P<0.05$) among the three groups was detected.
Descriptors: *Ceratothoa gaudichaudii*, ectoparasites, body weight, *Salmo salar*, PSW, Chile, Los Lagos, Guar Island, fish culture, isopoda, Chile, fish diseases
ASFA; Copyright © 2003, FAO

Stankovic B (1998) **Higijensko-sanitarni i zootehnicki standardi u uzgoju morske ribe. [Hygienic and zootechnical standards in marine fish farming.]** Ed: Vinkovic B. *The Proceedings of the 3rd Scientific Symposium on DDD with international participation. Let healthy stay healthy, Zadar, Croatia, 7-9 May, 1998.* Hrvatska veterinarska komora, Zagreb, Croatia. 293-296 (In Hungarian with English summary)

ISBN: 953-96576-4-4
Descriptors: disinfection, hygiene, marine fishes, animal welfare, Aquaculture

Stobo WT (1972) **Effects of formalin on the length and weight of yellow perch.** *Transactions of the American Fisheries Society.* 101(2):362-364
NAL Call No. 414.9 AM3
Data for fish growth studied often derive from formalin preserved specimens, but little information is available on the effect of the preservation on spiny-rayed fishes. The effect of 10% formalin on length and weight of yellow perch (*Perca flavescens*) was checked during an 18.5 months period. 55 perch were left in anaesthetic solution (0.8% ethylether) until death. Length and weight were recorded prior to death, after 1 hour, then after 1 hour in formalin. Observations were repeated on a geometric time scale for 1 week, weekly for 1 month, then monthly for 7 months and finally 18.5 months after killing. Tabled results show immediate shrinkage in small fish, largely complete in 24 hour but an initial increase in large fish with subsequent shrinkage complete in 5 days. No length corrections are thought necessary for preserved perch. In all perch weight showed an initial rapid increase, which slowed for a short period, then a protracted period of increase followed by a period of decrease (135 to 557 days).
ASFA; Copyright © 2003, FAO
Descriptors: formalin, yellow perch, spiny-rayed fishes, anesthetic

Waldbieser GC (1996) **Polymerase chain reaction amplification of genetic loci from diseased channel catfish found dead in ponds.** *Journal of Aquatic Animal Health.* 8(2):155-158
NAL Call No. SH171.J68
As part of a selective breeding program for farm-raised channel catfish *Ictalurus punctatus*, we screened diseased fish to identify genetic markers linked to disease resistance or susceptibility. Because many diseased fish in ponds are not detected until after death, we investigated the utility of DNA isolated from diseased channel catfish found dead in ponds. Channel catfish (4-25 g) diagnosed with enteric septicemia of catfish or saprolegniasis were sampled 24-48 h postmortem from infected ponds. Control fish were killed by anesthetic overdose and sampled immediately. Total DNA isolated from liver, muscle (with skin), and caudal fin was quantified and analyzed for degradation. Yield of purified DNA, measured as micrograms of DNA per milligram of tissue, was significantly ($P < 0.05$) lower in diseased fish than in controls. Two sets of DNA primers were used to amplify a portion of the channel catfish growth hormone gene and the mitochondrial D-loop region with the polymerase chain reaction. Degradation of DNA in liver and the caudal fin of some diseased fish inhibited successful amplification. Amplification of fragments up to 1,000 base pairs long from genomic DNA of post-mortem channel catfish will be useful for identifying molecular genetic markers linked to disease susceptibility.
Descriptors: selective breeding, disease resistance, Ictalurus punctatus, DNA, polymerase chain reaction, genetic markers, septicemia, saprolegniasis, growth hormone, USA, Mississippi
ASFA; Copyright © 2003, FAO

Wall T, Southgate PJ (1992) **Welfare of farmed fish.** *Veterinary Record* (England). 130(24):543
NAL Call No. 41.8 V641
Descriptors: animal welfare, fishes, animals, domestic, veterinary medicine, aquaculture

Wedekind C, Muller R, Steffen A, Eggler R (2001) **A low-cost method of rearing multiple batches of fish.** *Aquaculture.* 192(1):31-37
NAL Call No. SH1 A6
Experimental studies based on inferential statistics typically require the rearing of many batches of eggs or fish separately. If this is done with conventional fish rearing methods, the need for laboratory space and equipment are normally very high. This may prevent many researchers from experimentally approaching problems in fish genetics or ecology, especially if treatment differences are expected to be small. We have developed and successfully tested a new procedure with *Coregonus sp.* fry. Eggs were hatched in Petri dishes kept at 6°C. Yolk-sac fry were reared in a hanging bag system at 15°C with continuous water exchange. We estimate that our new fry-rearing method reduces space needs, infrastructure and material costs by a factor of 10 or more, while being comparable to previously described methods with respect to animal welfare requirements and the experimenter's working time.
Descriptors: aquaculture techniques, hatching, rearing, survival, experimental research, economics, costs, test organisms, fry
ASFA; Copyright © 2003, FAO

Web Resources:

Aspects Of Animal Welfare And Aquaculture -A Compendium of Selected Literature by Richard D. Moccia and Kristopher P. Chandroo; Aquaculture Centre, University of Guelph, Guelph, Ontario, Canada
http://www.aps.uoguelph.ca/~aquacentre/aec/publications/welfare-bib.html

Code of Environmental Management Practices: For Well Managed Salmonid Farms
Fundacion Chile
http://www.fisheries.org/CBPA%202003%20Ingl%E9s.pdf

CVM Guide 1240.4200
Low Regulatory Priority Aquaculture Drugs
http://www.fda.gov/cvm/index/aquaculture/LRPDrugs.pdf

CVM Guide 1240.4260
Classification of Aquaculture Species as Food or Nonfood
http://www.fda.gov/cvm/index/policy_proced/4260.pdf

Science Directorate Report of the Workshop on Farmed Fish Welfare
UK Department of the Environment, Food, and Rural Affairs
http://www.defra.gov.uk/science/Publications/Report%20of%20the%20Workshop%20on%20Farmed%20Fish%20Welfare.pdf
... a variety of morphological, physiological and histopathological indicators of fish welfare status ... 3. Review of scientific literature on effects on rainbow trout ...

Drugs Approved for Use in Aquaculture
http://www.fda.gov/cvm/index/aquaculture/appendixa6.htm

Fish Farming and Organic Standards
Briefing Paper
Soil Association
http://www.soilassociation.org/web/sa/saweb.nsf/librarytitles/Briefing_Sheets18102002.html

Fiskeriforkning Info No. 6 May 1999
Norwegian Institute of Fisheries and Aquaculture, Ltd. (NIFA)
http://www.fiskforsk.norut.no/0699e.pdf

Government Response To The Farm Animal Welfare Council's Report On The Welfare Of Farmed Fish
DEFRA, United Kingdom
http://www.defra.gov.uk/animalh/welfare/farmed/othersps/fish/fawc-fish/fawcftoc.htm

In Too Deep: The Welfare of Intensively Farmed Fish
A Report for Compassion in World Farming Trust
Philip Lymberly, 2001
http://www.eurocbc.org/itd10pg_In_too_deep_CIWFpdf.pdf

Pisces: Health and Welfare of Fish
http://www.Piscestt.com/Pisces/hottopics/healthandwf0_en.asp

Report on the Welfare of Farmed Fish
Farm Animal Welfare Council
http://www.fawc.org.uk/reports/fish/fishrtoc.htm

UC Davis –Gateway to Information on Optimal Care and Welfare of Fish
http://www.vetmed.ucdavis.edu/CCAB/fish.htm

Welfare of Farmed Fish
Pain and Fish Welfare
http://www.vet.ed.ac.uk/animalwelfare/Fish%20pain/welfare.htm#FARMED

3.2.3. AQUARIUM FISHES

3.2.3.1. General Topics

Deutsche Veterinarmedizinische Gesellschaft (1994) **Tagung der Fachgruppen "Tierschutzrecht und gerichtliche Veterinarmedizin" und "Fischkrankheiten", Stuttgart-Hohenheim, 10.-11. Marz 1994. [Proceedings of a meeting of the Expert Groups on animal protection law, veterinary jurisprudence and fish diseases, German Veterinary Medical Society, Stuttgart-Hohenheim, 10-11 March 1994].** Frankfurter Strasse 89, D-6300 Giessen, Germany. 111 pp. (In German with English Subtitles)
NAL Call No. SF605 T33 1994
The meeting discussed the welfare of fish kept in aquaria and of aviary birds.
Descriptors: animal welfare, aquarium fishes, aviary birds

Manz D (1988) **Tierschutzrelevante Gesichtspunkte bei der Haltung von Aquarienfischen. [Animal welfare aspects of the keeping of aquarium fish.]** *Tagung der Fachgruppe "Fischkrankheiten" und der Fachgruppe "Zootierkrankheiten" im Tierpark "Hellabrunn", Munchen, 22./ 23. November 1988.* Deutsche Veterinarmedizinische Gesellschaft Giessen, German Federal Republic. p.20-26 (In German)
NAL Call No. SF458.5 T3
Descriptors: animal welfare, aquarium fishes, fishes, aquatic animals

Weins J (1997) **Untersuchungen uber die Susswasserzierfischhaltung im Zoofachhandel unter tierhygienischen und tierschutzrechtlichen Aspekten mit Vorschlagen fur die Tatigkeit der berwachungsbehorden [Welfare aspects of the trade in freshwater aquarium fishes.]** Fachbereich Veterinarmedizin, Justus-Liebig-Universitat, Giessen Germany. 173 pp. (In German with English summary)
Descriptors: animal welfare, pets, aquarium fishes

Wildgoose W (2001) **Taking the plunge: treating pet fish**. *In Practice*. 23 (4):220-227
NAL Call No. SF601.I4
Descriptors: animal welfare, aquarium fishes, aquatic animals, fish diseases, nitrogen cycle, ornamental fishes, veterinarians, water filters, water quality, goldfish

Wilhelm W (1991) **The art of keeping aquarium fishes**. *Tijdschrift voor diergeneeskunde* (Netherlands). 116 Suppl 1 p. 57S-63S.
NAL Call No. 41.8 T431
Descriptors: animal welfare, fishes, physiology, animal feed, animal nutrition, housing, standard, water

3.2.3.2. Ornamentals

Biffar M (2002) **Recent developments in ornamental fish: market, health management and general trends.** *Bulletin of the European Association of Fish Pathologists.* 22 (2):66-71
ISSN: 0108-0288
Descriptors: animal health, animal welfare, aquarium fishes, hobbies, import controls, international trade, ornamental fishes, regulations

Ferraz E, Araujo MGL (1999) **Ornamental fish from the Rio Negro Basin: Overcoming disease related mortalities.** (Eds:) Nelson J, MacKinley D. *Special adaptations of tropical fish.* pp. 11-16
Brazil is a major exporter of aquarium fish. The main source for ornamental fish for export is the Rio Negro basin. The techniques for fish capture and transportation are, basically, the same as those used at the beginning of exploitation. Though some improvements have been made in fish husbandry management post-capture, diseases and mortalities are still a problem. A variety of approaches were used to assess the health problems: 1) interviews with fishermen, middlemen and exporters; 2) recording the condition of the fish on their arrival at the exporters' holding facilities; and 3) routine disease screening of 7 species of fish. Results indicate: 1) that the seasonal fluctuations of the water level play an important role in determining fish health; fish captured from areas with low water levels during the long dry season are often found to be in a debilitated condition; 2) in the chain of events linking capture to export the most critical phases influencing the health and survival of the fish are the storage of fish by fishermen and the transport to the local reception areas of the exporters in Barcelos. The transport from Barcelos to the principal fish holding facilities in Manaus was previously considered a critical phase, but the recent provision of new boats for fish transport, and improvements in fish husbandry prior to transport, have contributed to reduced mortalities during this period; 3) the most common diseases diagnosed are related to the series of handling stressors and sub-optimal conditions which fish are exposed to prior to delivery at the exporters' holding facilities. The negative effects of handling stress on the condition of the fish can be reduced if more attention is paid to the welfare of the fish following the arrival of the consignments at the reception areas in Barcelos. Another problem concerning the Brazilian ornamental fish industry concerns the legislation controlling the export of ornamental species. Problems related to the export of non-permissible species possessing similar external characteristics to and recorded under the same Latin or common name as a permissible species, are still present.
Descriptors: ornamental fish, tropical fish, diseases, mortality, trade, fish handling, transportation, live storage, river basins, *Paracheirodon axelrodi, Hyphessobrycon erythrostigma, Symphysodon discus, Ancistrus, Corydoras robinae, Corydoras adolfoi, Corydoras burguessi,* Brazil

Ford, DM (1991) **Ornamental fish**. Eds: Anderson RS, Edney ATB. *Practical animal handling.* Pergamon Press plc Oxford, UK. p.171-176
NAL Call No. SF61 P73 1991
Topics discussed are the inspection tank, the fresh water tank, salt water tank, maintenance, examination, the home aquarium, the pond fish and capture and transporting of fish.
Descriptors: fish diseases, transport of animals, restraint of animals, animal welfare

Hignette M (1984) **The use of cyanide to catch tropical marine fish for aquariums and its diagnosis.** Comptes Rendus des Journees Aquariologiques de l'Institut Oceanographique, 16 Dec 198 (Proceedings of Marine Aquariology of the Oceanographical Institute, 16 Dec 1983.). *Oceanis. Serie de documents oceanographiques. Paris.* 10(5):585-591
ISSN: 0182-0745
Marine tropical fish for the pet industry are nowadays very often caught with cyanide sodium, which is used for its "anaesthetic" effect. This method however can be responsible for fish dying as much as several weeks after transport, and must be avoided. In order to stop exporters having recourse to this practice, fish importers and aquariologists must know how to measure cyanide themselves in fish or obtain analyses from reliable laboratories.
Descriptors: cyanides, aquaria, tropical fish, catching methods, fish poisoning, stupefying methods, mortality causes
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Wyllie S (1994) **The international ornamental fish trade**. *State Veterinary Journal.* 4(1):11-13
NAL Call No. SF601 S8
Descriptors: ornamental fishes, transport of animals, animal welfare, regulations, aquaria, trade, collections, transport

Web Resources:

Marine Aquarium Council
http://www.aquariumcouncil.org/default.asp

Ornamental Fish
http://www.ornamentalfish.org/association/code/conduct/conduct.php
Provides code of conduct that addresses all aspects of ornamentals.

3.2.3.3. Dealers & Pet Shops

Chan TTC, Sadovy Y (1998) **Profile of the marine aquarium fish trade in Hong Kong.** *Aquarium Sciences and Conservation.* 2(4):197-213

A market survey and review of government statistics were carried out to establish imports and exports of marine ornamental fishes into and out of Hong Kong, and to examine the local trade in terms of volume, value and species composition. Official government import figures for marine aquarium fishes were available from 1984 to 1991 and from 1997 and 1998. When compared with net imports, export and re export volumes over the 15 year period were small, indicating that most imports entered the local market, or were exported unrecorded. The market survey of marine aquarium shops in Hong Kong was carried out between August 1996 and January 1997. From this survey, an annual estimate of 957,563 coral reef fish was calculated for the local trade, valued at HK$57,453,780, with a mean retail price of HK$60 per fish. These figures account for an estimated 2 3% of the global value and volume of marine aquarium fish trade, according to 1992 figures and, compared with government figures, indicate that official declarations of imports are underreported by at least 2 3 fold. A total of 342 marine aquarium fish species, from 49 families, were recorded with about 60% belonging to the families Labridae, Chaetodontidae, Pomacanthidae and Pomacentridae. It was estimated that a large proportion of the trade was in juvenile fishes and almost all fish were less than 10 cm in standard length. It was common to see fishes in poor condition, species hard to maintain in captivity, or those listed for conservation concern, on sale. There are no regulations for local traders of live marine fishes other than those relating to either protected species or animal welfare. Licensing of traders in marine fishes could be introduced under existing Hong Kong legislation if fishes were to be re classified as animals. This is strongly recommended as a step towards regulating and managing the trade in Hong Kong.

Descriptors: aquarium culture, ornamental fish, aquaculture economics, marine fish, resource conservation, ISEW, Hong Kong

Etscheidt J (1995) **Kriterien zur beurteilung der haltung von suesswasseraquarienfischen im zoohandel [Critical aspects of the welfare of freshwater fish in pet shops].** *Tieraerztliche Umschau.* 50(3):196-199 (In German)

NAL Call No. 41.8 T445

It is possible with the aid of key parameters to assess the welfare of freshwater fish kept in petshops. The most important factors are water quality, stocking density, social behaviour, inter-species interactions, the decoration and structure of the fish tanks, the technical devices and the expertise of the owner in terms of feeding and the management of water changes and filtering.

Descriptors: freshwater fish, ornamental fish, stocking density, water quality control, aquariology, fish, animal welfare

Nowak, D. (1993) **Tierschutzrelevante Probleme bei der Kontrolle von Zoofachgeschaften aus amtstierarztlicher Sicht [Problems of animal welfare during official veterinary surveillance of pet shops].** *Deutsche Tierarztliche Wochenschrift* 100(2):76-78 *(In German with English Summary)*

NAL Call No. 41.8 D482

From a survey of all official veterinarians in Berlin. Typical problems of keeping fishes, birds, rodents and reptiles are listed. Many of the vets reported the lack of practical guidance, manuals or expert opinions.
Descriptors: pets, aviary birds, animal welfare, veterinary services
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Winter M (1993) **Tierschutzgerechte Haltung von Zierfischen im Zoohandel - eine Studie. [Humane handling of aquarium fish by animal dealers.]** Tierarztliche Fakultat, Ludwig-Maximilians-Universitat, Munchen. 190 pp. (In German with English summary). Thesis.
Descriptors: zootechny, animal welfare, aquarium fishes, fishes
Copyright © 2003, CAB International.

Web Resources:

APHIS Animal Care Publications
http://www.aphis.usda.gov/ac/publications.html
Links to the Animal Welfare Act, Regulations, and Standards and other related publications.

3.2.4. FISHERIES

Gregory NG (2000) **Animal welfare in the fish industry.** *Surveillance (Wellington).* 27(2):8-10
NAL Call No. SF604.63.N45S87
Descriptors: animal welfare, fisheries, aquaculture, stress, fishing, fish farming, fishes, aquatic animals

Joll C (1992) **Fishermen encouraged to enter animal welfare debate.** *Australian Fisheries.* 51(10):19-20
NAL Call No. SH317 A8
The animal welfare debate has moved into the arena of fisheries. Malcolm Cleland, a farmer and consultant, suggests that fishermen should lay down plans now to protect themselves against criticisms that are likely to emerge within this decade. Fishermen have been urged to acquaint themselves with the animal welfare issues under debate and to initiate a code of practice for themselves, so they will be better placed to deflect criticism of fishing practices that industry considers acceptable. The advice has come from Malcolm Cleland, a Tasmanian sheep farmer, who has been active in the animal welfare debate for more than 10 years. Although not an animal liberationist himself, he maintains that the debate is here to stay. He says primary producers--including fishermen--cannot afford to ignore it.
Descriptors: fishing operations, fishery disputes, fishery industry, fishery resources, Australia
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Schulz U (1995) **Elektrofischerei unter Tierschutzaspekten. [Animal welfare aspects of electrofishing.]** *Deutsche Tierarztliche Wochenschrift.* 102 (3):125-127 (In German with English summary)
NAL Call No. 41.8 D482
Descriptors: legislation, fish farming, fishing, electricity, animal welfare, fishes

Seemann (1978) **Fischerei und Tierschutz. [Fisheries and animal welfare].** *Du und das Tier.* 8(1):10-11 (In German)
ISSN: 0341-5759
Descriptors: animal welfare, pain, angling, fishes

Spitler RJ (1998) **The animal rights movement and fisheries**. *Fisheries* (Bethesda). 23(1):21-22
NAL Call No. SH1.F54
Descriptors: animal welfare, ethics, fishing, public opinion, animal sports, fishes

Web Resources:

Rotenone Use in Fisheries Management: Administrative and Technical Guidelines Manual American Fisheries Society
http://www.fisheries.org/rotenone/Rotenone_Manual.pdf

Schramm, H. L., Jr., and R. G. Piper, editors. 1995. **Uses and Effects of Cultured Fishes in Aquatic Ecosystems.** *American Fisheries Society Symposium 15*, Bethesda, Maryland, USA. (http://www.fisheries.org) ISBN: 0-913235-91-1
http://www.fisheries.org/publications/catbooks/uecf.htm

3.2.5. LABORATORY

Bakken M, Vangen O, Rauw WM (1998) **Biological limits to selection and animal welfare.** *Proceedings of the 6th World Congress on Production, Armidale, Australia, January 11-16, 1998. Volume 27: Reproduction; fish breeding; genetics and the environment; genetics in agricultural systems; disease resistance; animal welfare; computing and information technology; tree breeding.* World Congress on Genetics Applied to Livestock Production. Armidale, Australia. p.381-388
NAL Call No. SF105 W67 1998
This paper discusses the relationships between selection of livestock for improved production, the biological limits to selection and animal welfare. Consideration is given to (1) the definition of animal welfare, (2) the application of resource allocation theory to study the relationships between animal welfare and artificial selection, (3) experiments on selection limits in laboratory animals, and (4) undesirable correlated responses resulting from selection for performance in poultry and farm animals.
Descriptors: selection, animal welfare, livestock

Blackshaw JK, Allan DJ, Martin AA, Tyler MJ (1987) ***Principles of Laboratory Animal Management-3rd ed.*** N.S.W., Australia: Australian Society for the Study of Animal Behaviour 103 p.
NAL Call No. SF406.B5 1987
Descriptors: laboratory animals, animal welfare

Bourdon RM, Enns RM (1998) **Physiological breeding values: rethinking the way we express genetic values for improving production systems.** *Proceedings of the 6th World Congress on Genetics Applied to Livestock Production, Armidale, Australia, January 11-16, 1998. Volume 27: Reproduction; fish breeding; genetics and the environment; genetics in agricultural systems; disease resistance; animal welfare; computing and information technology; tree breeding.* World Congress on Genetics Applied to Livestock Production. Armidale, Australia. 227-234
NAL Call No. SF105 W67 1998
This paper is concerned with the determination of breeding objectives for different combinations of management systems and biotypes in order to arrive at the optimal combination of management system and breeding objectives. Bioeconomic simulation is suggested as a technique for evaluating production systems. The genetic inputs to a bioeconomic simulation model should satisfy 2 criteria: (1) they must indicate the underlying genetic potential, i.e. genetic potential that is not compromised by the constraints commonly found in field data; (2) they must measure traits that are logical inputs for mechanical modelling. Physiological breeding values (PBV) are such inputs. PBV are similar to conventional breeding values, except that the definition of genetic potential is different. PBV are independent of population and environment but dependent on the model used. The problems associated with estimating PBV from conventional breeding values are discussed in relation to body weight and milk yield in cattle.
Descriptors: breeding value, production, systems, models, livestock, fishes

Casebolt DB, Speare DJ, Horney BS (1998) **Care and use of fish as laboratory animals: current state of knowledge.** *Laboratory animal science* (United States). 48(2):124-36
NAL Call No. 410.9 P94
Descriptors: animal welfare, animals, laboratory, fishes, genetically modified, genetic engineering, veterinary

Chen TT, Lu JK, Dunham RA, Powers DA (1994) **Transgenic fish: Ideal models for basic research and biotechnological application.** *3rd International Marine Biotechnology Conference: Program, Abstracts And List Of Participants.,* Tromsoe University, Tromsoe (Norway), p. 70.
A wide range of transgenic animal species including fish have been produced by microinjecting or electroporating transgenes into developing embryos. This technology offers excellent opportunities for creating model animals for basic research as well as biotechnological applications. In basic research, transgenic animals provide excellent experimental models for studying molecular genetics of early vertebrate development, actions of oncogenes, and the biological actions of hormones at different stages of development. In applied biotechnology, transgenic technology offers unique opportunities for producing animal models for research in biomedical problems and environmental toxicology, improving the genetic background of broodstock for animal husbandry or aquaculture, and designing bioreactors for producing valuable proteins for pharmaceutical or industrial purposes. We have been studying the biological actions of growth hormone and insulin-like growth factor as well as concept of molecular toxicology, using transgenic common carp, channel catfish and medaka as experimental animals. In this paper we will use results from our own studies as well as these from others to demonstrate the potential application of transgenic fish technology.
Descriptors: transgenic animals, Pisces, research programs, biotechnology, models, genetics, genomes, aquaculture techniques
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DeTolla LJ, Srinivas S, Whitaker BR, Andrews C, Hecker B, Kane AS, Reimschuessel R (1995) **Guidelines for the care and use of fish in research.** *ILAR Journal* 37(4):159-173.
NAL Call No. QL55.A1I43
Descriptors: fishes, laboratory animals, animal experiments, animal husbandry, animal welfare, medical research, anesthesia, anesthetics, euthanasia, zoonoses, guidelines, regulations

Goolish EM, Evans R, Okutake K, Max R (1998) **Chamber volume requirements for reproduction of the zebrafish *Danio rerio*.** *Progressive Fish Culturist.* 60(2):127-132
NAL Call No. 157.5 P94
The zebrafish or zebra danio *Danio (=Brachydanio) rerio* has recently become a major vertebrate model for the study of developmental biology, neurobiology, and molecular genetics. As a result, most research universities have now invested considerable resources in the construction of large zebrafish facilities. A key element in the design of these facilities is maximizing the efficiency of available space. Here we report on the effects of aquarium chamber volume on the reproduction of zebrafish, with the objective of identifying the minimal volume required for normal egg production. Six adults (two males and four females) were tested in chamber volumes of 500, 400, 300, 200, and 100 mL. Results were compared with those from a control volume of 3.5 L. Eggs were removed from the test chambers after spawning and incubated in petri dishes at 28°C. Total egg production, percent of eggs

hatching, and larval length at 96 h postfertilization were used to evaluate breeding success. Compared with the control, egg production was not significantly affected by reduced aquaria volumes of 500, 400, and 300 mL. However, mean egg production from a test volume of 200 mL was only 48% of the control egg production ($P < 0.05$), and at a test volume of 100 mL, egg production was reduced to 26% of the control value ($P < 0.005$). Percent egg hatch and 96-h larval length were not affected at any test volume.
Descriptors: laboratory culture, test organisms, aquaculture facilities, stocking density, *Danio rerio*, Zebra danio
ASFA; Copyright © 2003, FAO

Grunwald DJ, Eisen JS (2002) **Headwaters of the zebrafish - emergence of a new model vertebrate.** *Nature Reviews: Genetics.* 3(9):717-724
ISSN: 1471-0056
The understanding of vertebrate development has advanced considerably in recent years, primarily due to the study of a few model organisms. The zebrafish, the newest of these models, has risen to prominence because both genetic and experimental embryological methods can be easily applied to this animal. The combination of approaches has proven powerful, yielding insights into the formation and function of individual tissues, organ systems and neural networks, and into human disease mechanisms. Here, we provide a personal perspective on the history of zebrafish research, from the assembly of the first genetic and embryological tools through to sequencing of the genome.
Descriptors: reviews, animal models, embryogenesis, models, genetics, embryology, research, historical account, *Danio rerio*, Zebra danio
ASFA; Copyright © 2003, FAO

Ivetac I, Becanovic J, Krishnapillai V (2000) **Zebrafish: Genetic tools and genomics.** *Asia-Pacific Journal of Molecular Biology and Biotechnology.* 8(1):1-11
ISSN: 0128-7451
The emergence of zebrafish (*Danio rerio*) as a model organism with applications in vertebrate developmental genetics, modeling and study of human genetic diseases, study of vertebrate genome evolution and improved aquaculture of transgenic fish, has been accompanied by the development of a vast array of zebrafish-specific genetic tools and genomic resources. Owing to its amenability to both phenotypic analysis and mutational screening and availability of a multitude of molecular genetic techniques, numerous zebrafish mutations have been assayed, extensive genetic maps created and many genes cloned. Comparative genomics using mammalian genomes is important to the provision of candidate genes for positional cloning strategies and gaining further insights into vertebrate genome evolution. With the likely sequencing of the entire zebrafish genome in the foreseeable future and further identification and cloning of zebrafish gene loci, the assignment of functions to uncharacterised human genes, known only by sequence from the Human Genome Project, is likely to become a reality.
Descriptors: reviews, genetic mapping, genomics, test organisms, genetics, laboratory culture, genomes, biological development, morphogenesis, organogenesis, *Danio rerio*, Zebra danio, genetic mapping
ASFA; Copyright © 2003, FAO

Khudoley VV (1984) **Use of aquarium fish, *Danio rerio* and *Poecilia reticulata*, as test species for evaluation of nitrosamine carcinogenicity.** *Use of Small Fish Species in Carcinogenicity Testing., Monograph Series.* National Cancer Institute. 65:65-70

The carcinogenic effects of various doses of dimethylnitrosamine (DMNA), diethylnitosamine (DENA), and mitrosomorpholine (NM), as well as the results of varying temperature and length of exposure to these carcinogens, were studied in short-term (20-21 wk) experiments in aquarium fish (820 *Danio rerio* and 944 *Poecilia reticulata*). All nitroso compounds induced liver tumors and esophageal papillomas. Exposure to NM also induced intestinal adenocarcinomas in *D. rerio*. When exposure time was shortened to 2 weeks, the dosage was decreased, or if the temperature was reduced to 17° ± 1° C, the tumor incidence dropped and the latency increased. An increase in temperature to 27° ± 1°C resulted in a 72-89% tumor incidence and shortened the latency to 11.3-14.1 weeks.
Descriptors: test organisms, nitrosamines, carcinogenesis, carcinogenicity testing, N-nitrosodiethylamine, N-nitrosodimethylamine, N-nitrosomorpholine, laboratory testing, carcinogenesis, *Danio rerio, Poecilia reticulata*, aquatic organisms
ASFA; Copyright © 2003, FAO

Klontz, GW (1995) **Care of fish in biological research.** *Journal of Animal Science.* 73(11): 3485-3492.
NAL Call No. 49 J82
Fish live in a very complex, dynamic environment. Their use as biological research subjects during the past three decades has increased almost exponentially because of the demand for an increased knowledge base in response to the need for better aquaculture technology. To use fish as biological research subjects requires the investigator to take into account approximately 40 interactive environmental variables, if the research data are to be free of unwanted biases. These environmental factors are classified into five major groups, all important to the well-being of fish. These five include intrinsic factors (fish associated) and extrinsic factors (water, container, nutrition, and management associated). The stress response is the primary intrinsic factor of concern, and associated pathological changes should be used to monitor animal well-being and prevent secondary infectious disease problems. The water-associated factors are the primary extrinsic factors affecting the well-being of fish. Thus, the investigator must design research protocols that maintain fish within documented environmental limits for the species.
Descriptors: fishes, fish culture, environmental control, water quality, animal welfare, fish feeding

Lin S, Yang S, Hopkins N (1994) **LacZ expression in germline transgenic zebrafish can be detected in living embryos.** *Developmental Biology.* 161(1):77-83
NAL Call No. 442.8 D49
Use of transgenic technology in zebrafish (*Brachydanio rerio*) has been limited by the inability to efficiently express transgenes in early embryos of F1 and subsequent generations and to rapidly detect transgenic fish. We generated transgenic fish by injecting fertilized eggs with the *Escherichia coli* lacZ gene under the control of the *Xenopus* elongation factor 16a transcriptional regulatory element. Four of five lines of transgenic fish we obtained express the lacZ gene in early embryos. The pattern of expression was distinct for each line, with two lines showing extensive expression beginning at approximately the midblastula transition, one showing patchy expression and one showing expression almost exclusively in motor neurons. Expression patterns were stable through the F2 generation in the three lines studied to date. The availability of these lines facilitated the development of a reliable and rapid method for live-staining lacZ-expressing embryos using the substrate fluorescein-di- beta -D-galactopyranoside (FDG). Positive embryos of the two most highly lacZ-expressing lines could be identified after 2-3 min of staining in FDG and then picked out and raised. These

observations should prove useful for a variety of studies in zebrafish.
Descriptors: transgenic animals, *Danio rerio*, production, use, lacZ gene, germ cells, gene expression, embryos, genes, experimental research, genetics, biotechnology, *Brachydanio rerio*, transgenic zebrafish

McHugh Law J (2001) **Mechanistic considerations in small fish carcinogenicity testing.** *ILAR Journal* 42(4): Fish Models in Biomedical Research.
NAL Call No. QL55 A1I43
URL: http://dels.nas.edu/ilar/jour_online/42_4/mechanistic.asp
Historically, small fish species have proven useful both as environmental sentinels and as versatile test animals in toxicity and carcinogenicity bioassays. They can be bred in large numbers, have low maintenance and bioassay costs, and have a low background incidence of tumors. However, more mechanistic information is needed to help validate the information garnered from these models and to keep pace with other more fully developed animal models. This paper focuses on mechanistic considerations when using small fish models for carcinogenicity testing. Several small aquarium fish species have proven useful. The Japanese medaka is perhaps the best characterized small fish model for carcinogenicity testing; however, the zebrafish is emerging as an important model because it is well characterized genetically. Both route and methodology of exposure may affect the outcome of the study. Most studies have been conducted by introducing the test compound into the ambient water, but dietary exposures and embryo microinjection have also been used. Other considerations in study design include use of an initiating carcinogen, such as diethlynitrosamine, and differences in xenobiotic metabolism, such as the fact that fish CYP2B is refractory to Phenobarbital induction. The small size of these models has perhaps limited some types of mechanistic studies, such as formation and repair of DNA adducts in response to carcinogen exposure. However, improved analytical methods are allowing greater resolution and should be applied to small fish species. Slide-based methods such as immunohistochemistry are an important adjunct to routine histopathology and should be included in study design. However, there is a need for development of more species-specific antibodies for fish research. There is also a need for more fish-specific data on cytokines, serum biochemistry, and oncogenes to strengthen the use of these important test models.
Descriptors: fish models, human disease, biomedical research, carcinogenicity, cytochrome P450, diethylnitrosamine, DNA adduct, hepatocarcinogenesis, medaka, small fish models, zebrafish
Note: Abstract reprinted with permission from ILAR Journal, Institute for Laboratory Animal Research, National Academy of Sciences, 500 Fifth Street, NW, Washington, DC 20001 (http://dels.nas.edu/ilar). To purchase copies of this article and the entire issue in which it appears, email <ilarj@nas.edu>.

Midtlyng PJ (1997) **Novel vaccines and new vaccination strategies for fish.** *Bulletin of the European. Association of Fish Pathologists.* 17(6):239-244
ISSN: 0108-0288
Sixty years after the first attempts to vaccinate fish, immunoprophylaxis has become the dominant strategy for disease control in commercial fish farming. Successful immunisation programmes against bacterial diseases of salmon leave little need for chemotherapy, thus providing pioneer evidence how the use of antimicrobials in industrial animal production can be curbed. The population of farmed fish now equals the numbers in other segments of world animal production, and aquaculture is rapidly moving into the focus f the international

agrochemical and pharmaceutical industry. At the same time, fish immunology and fish vaccinology from an academic discipline of pioneers to a commercially driven biotechnology and biomedical science. The present paper attempts to summarise the scientific contributions of the IABS symposium on Fish Vaccinology, which took place in Oslo, Norway June 5-7 1996, and from which the proceedings volume containing 45 review articles recently have been published. Among the subjects covered are recent achievements in research on fish immune mechanisms; development of vaccines against bacterial, viral and parasitic diseases of fish; issues relative to the production, evaluation and licensing of fish vaccines; safety and environmental issues; and recommended vaccination strategies for various aquacultured species and productions.
Descriptors: vaccination, fish culture, disease control
ASFA; Copyright © 2003, FAO

Midtlyng PJ (2001) **Vaccination of fish - achievements and challenges.** *NATO Science Series: Series A: Life Sciences. Modern Aquaculture in the Coastal Zone-Lessons and Opportunities.* 314:197-211.
ISSN: 1387-6686
The first written scientific communications of fish vaccination are now more than 60 years old, and immunoprophylaxis has become the dominant strategy for disease control in commercial fish farming. Successful immunization programmes against bacterial diseases of salmon have dramatically diminished the need for chemotherapy, thus providing pioneer evidence how the use of antimicrobials in industrial animal production can be reduced. The population of farmed fish has now surpassed other segments of world animal production in numbers, and aquaculture is rapidly moving into the focus of the international agrochemical and pharmaceutical industry. At the same time, fish immunology and fish vaccinology has evolved from an academic discipline of pioneers to a commercially driven biotechnology and biomedical science. This paper presents an overview of immunoprophylaxis in current fish farming, giving some examples of current opportunities and practices of vaccination in European coastal aquaculture. Recent achievements in research on fish immune mechanisms; in the development of vaccines against bacterial, viral and parasitic diseases of fish; issues relative to the production, evaluation and licensing of fish vaccines; safety and environmental issues; and recommended vaccination strategies for various aquacultured species and productions are being outlined.
Descriptors: vaccines, vaccination, fish diseases, disease control, Pisces, salmonids
ASFA; Copyright © 2003, FAO

Moorman SJ (2001) **Development of sensory systems in zebrafish (*Danio rerio*).** *ILAR Journal* 42(4): Fish Models in Biomedical Research.
NAL Call No. QL55 A1I43
URL: http://dels.nas.edu/ilar/jour_online/42_4/sensory.asp
Zebrafish possess all of the classic sensory modalities: taste, tactile, smell, balance, vision, and hearing. For each sensory system, this article provides a brief overview of the system in the adult zebrafish followed by a more detailed overview of the development of the system. By far the majority of studies performed in each of the sensory systems of the zebrafish have involved some aspect of molecular biology or genetics. Although molecular biology and genetics are not major foci of the paper, brief discussions of some of the mutant strains of zebrafish that have developmental defects in each specific sensory system are included. The development of the sensory systems is only a small sampling of the work being done using

zebrafish and provides a mere glimpse of the potential of this model for the study of vertebrate development, physiology, and human disease.
Descriptors: fish models, human disease, biomedical research, dorsal root ganglion, inner ear, lateral line, olfactory system, vestibular system, visual system
Note: Abstract reprinted with permission from ILAR Journal, Institute for Laboratory Animal Research, National Academy of Sciences, 500 Fifth Street, NW, Washington, DC 20001 (http://dels.nas.edu/ilar). To purchase copies of this article and the entire issue in which it appears, email <ilarj@nas.edu>.

Mottet NK, Landolt ML (1987) **Advantages of using aquatic animals for biomedical research on reproductive toxicology.** *Environmental Health Perspectives. 71:69-75*
NAL Call No. RA565 A1E54
Major advantages of the use of aquatic animals, such as trout (*Salmo gairdneri*), English sole (*Parophrys vetulus*), or sea urchins (*Strongylocentrotus purpuratus*), for studying the mechanisms of reproductive toxicology are discussed. The remarkable synchrony of differentiation of gametes in large quantities for detailed morphologic and biochemical measurements enables research not readily done on mammalian nonseasonal breeders. Structural differences such as the absence of a fibrous sheath in the more simple structure of fish and sea urchin sperm flagella facilitates comparative study of the mechanism of action of microtubules in flagella movement and the coupling of mitochondrial energy production to microtubules movement.
Descriptors: toxicology, reproduction, animal models, aquatic animals, toxicants, sexual reproduction, aquatic organisms, models, *Salmo gairdneri, Parophrys vetulus, Strongylocentrotus purpuratus*, mechanisms
ASFA; Copyright © 2003, FAO

Ostrander GK (2000) ***Laboratory Fish.*** Academic Press, San Diego, CA. 678p
ISBN: 0-12-529650-9
Descriptors: animals, laboratory; fishes; fish as laboratory animals.

Reimschuessel R (2001) **A fish model of renal regeneration and development.** *ILAR Journal* 42(4): Fish Models in Biomedical Research.
NAL Call No. QL55 A1I43
URL: http://dels.nas.edu/ilar/jour_online/42_4/fish_model.asp
The fish kidney provides a unique model for investigating renal injury, repair, and development. Like mammalian kidneys, fish kidneys have the remarkable ability to repair injured nephrons, designated renal regeneration. This response is marked by a recovery from acute renal failure by replacing the injured cells with new epithelial cells, restoring tubule integrity. In addition, fish have the ability to respond to renal injury by de novo nephron neogenesis. This response occurs in multiple fish species including goldfish, zebrafish, catfish, trout, tilapia, and the aglomerular toadfish. New nephrons develop in the weeks after the initial injury. This nephrogenic response can be induced in adult fish, providing a more abundant source of developing renal tissue compared with fetal mammalian kidneys. Investigating the roles played by different parts of the nephron during development and repair can be facilitated using fish models with differing renal anatomy, such as aglomerular fish. The fish nephron neogenesis model may also help to identify novel genes involved in nephrogenesis, information that could eventually be used to develop alternative renal replacement therapies.

Descriptors: fish models, human disease, biomedical research, development, fish, kidney, model, nephron, regeneration, repair
Note: Abstract reprinted with permission from ILAR Journal, Institute for Laboratory Animal Research, National Academy of Sciences, 500 Fifth Street, NW, Washington, DC 20001 (http://dels.nas.edu/ilar). To purchase copies of this article and the entire issue in which it appears, email <ilarj@nas.edu>.

Smigielski AS (1975) **Hormone-induced spawnings of the summer flounder and rearing of the larvae in the laboratory.** *Progresive Fish Culturist.* 37(1):3-8
NAL Call No. 157.5 P94
Adult and immature summer flounder (*Paralichthys dentatus*) captured in Narragansett Bay and Block Island Sound were transported to the Northeast Fisheries Center, Narragansett Lab, Narragansett, Rhode Island, in 1970-71 and 1972. A photoperiod of 11 hr light 13 hr dark (11L:13D) was maintained by time clocks for 3 seasons, and water temps in the observation aquarium were allowed to fluctuate and coincide with bay temps during the seasons of 1970 and 1971. During the season of 1972, water temps in the observation aquarium and smaller fiberglass holding tank were maintained at 18° ± 1°C. The size and strength of the adult summer flounder necessitated the application of anaestesia before administering hormone injections or spawning the fish. The anaesthetic Tricaine Methane Sulfonate (MS-222) at a conc of 1:20,000 was used without any harmful effects. Carp pituitary (freeze-dried powder) administered at the dosage levels of 0.5 mg and 5.0 mg/454 g fish caused hydration and subsequent ovulation at both dosage levels. The low fertilization percentages of eggs experienced in the lab resulted from an insufficient number of running ripe males readily available. It is hoped that in the future a major effort will be made to obtain and maintain larger numbers of ripe males in the lab. The eggs obtained from these hormonal induced spawnings appeared to be normal in all respects. They had an average diam of 1.02 mm and contained 1 large oil globule which averaged 0.25 mm. Incubation was carried out at 2 temps 15° and 18°C. Hatching occurred between 72 and 96 hr at a salinity of 32 ppt. Larvae obtained from hormone-induced spawnings appeared to be normal in all respects and no abnormalities were noted. Their survival percentage was greater at water temps of 15°C and when reared in black-sided aquariums as opposed to clear-sided aquaruims. Survival was also greater when a Chlorella sp like algae was introduced into their rearing aquariums regardless of the colour background. Appropriate sixed zooplanktons, introduced at the proper time and amounts, are extremely important in rearing summer flounder larvae. It is hoped that in the future food density studies can be carried out to determine optimum food levels necessary for survival for this sp. As far as is known, these are the first successful hormone-induced spawnings reported with summer flounder and the subsequent rearing of their larvae through metamorphosis in the lab. The entire early life cycle of this flat fish can now be completed under controlled lab conditions.
Descriptors: induced breeding, hormones, rearing, fish larvae, *Paralichthys dentatus*

Stoskopf MK (2002) **Biology and health of laboratory fishes**. Eds: Fox JG, Anderson LC, Loew FM, Quimby FW. *Laboratory animal medicine* (Ed 2):886-907. Academic Press London, UK
NAL Call No. SF996.5 L33
Descriptors: animal health, animal welfare, bacterial diseases, biology, laboratory animals, fishes

Stoskopf M (2001) **Introduction.** Fish Models in Biomedical Research. *ILAR Journal* 42(4):
NAL Call No. QL55 A1I43
URL: http://dels.nas.edu/ilar/jour_online/42_4/introduction.asp
Descriptors: fish models, human disease, biomedical research

Vogl C, Grillitsch B*, Wytek R, Spieser OH, Scholz W (1999) **Qualification of spontaneous undirected locomotor behavior of fish for sublethal toxicity testing Part I. Variability of measurement parameters under general test conditions.** *Environmental Toxicology and Chemistry.* 18(12):2736-2742
NAL Call No. QH545.A1E58
An automated, personal computer-based video-processing, object-recognition, and object-tracing system was used to record and analyze undirected spontaneous locomotor behavior of small groups of undisturbed semiadult zebra fish (*Brachydanio rerio*) in laboratory tanks. The primary data provided by the monitoring system were the individually assigned, time-stamped coordinates of the fish in two-dimensional projection. Secondary parameters (position, velocity of movement in the horizontal and the vertical direction, and temporal intraindividual and interindividual association) were calculated. The computed parameters offered a multidimensional description of spontaneous undirected swimming behavior of the fish and proved to be largely independent of water temperature, length, weight, and sex ratio of the zebra fish within the standardized range, but varied significantly with the feeding regime, time of day, number of fish per tank, and batch. Statistical characteristics of the behavioral parameters confirmed them as being appropriate for parametric statistical analyses.
Descriptors: toxicity testing, behavior, aquatic organisms, computer applications, laboratory methods, Pisces, statistical analysis, monitoring systems, *Brachydanio rerio*, locomotor activity, swimming behavior, toxicity tests, bioaccumulation, pollution surveys, indicator species, swimming, sex ratio, toxicity, testing procedures, fish, bioindicators, water temperature, *Danio rerio*, statistical analysis, Zebra danio
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Walter RB, Kazianis S (2001) ***Xiphophorus* interspecies hybrids as genetic models of induced neoplasia.** *ILAR Journal* 42(4): Fish Models in Biomedical Research.
NAL Call No. QL55 A1I43
URL: http://dels.nas.edu/ilar/jour_online/42_4/hybrids.asp
Fishes of the genus *Xiphophorus* (platyfishes and swordtails) are small, internally fertilizing, livebearing, and derived from freshwater habitats in Mexico, Guatemala, Belize, and Honduras. Scientists have used these fishes in cancer research studies for more than 70 yr. The genus is presently composed of 22 species that are quite divergent in their external morphology. Most cancer studies using *Xiphophorus* use hybrids, which can be easily produced by artificial insemination. Phenotypic traits, such as macromelanophore pigment patterns, are often drastically altered as a result of lack of gene regulation within hybrid fishes. These fish can develop large exophytic melanomas as a result of upregulated expression of these pigment patterns. Because backcross hybrid fish are susceptible to the development of melanoma and other neoplasms, they can be subjected to potentially deleterious chemical and physical agents. It is thus possible to use gene mapping and cloning methodologies to identify and characterize oncogenes and tumor suppressors implicated in spontaneous or induced neoplasia. This article reviews the history of cancer research using *Xiphophorus* and recent developments regarding DNA repair capabilities, mapping, and

cloning of candidate genes involved in neoplastic phenotypes. The particular genetic complexity of melanoma in these fishes is analyzed and reviewed.
Descriptors: fish models, human disease, biomedical research, CDKN2, melanoma, mnu, platyfish, swordtail, UV, Xmrk
Note: Abstract reprinted with permission from ILAR Journal, Institute for Laboratory Animal Research, National Academy of Sciences, 500 Fifth Street, NW, Washington, DC 20001 (http://dels.nas.edu/ilar). To purchase copies of this article and the entire issue in which it appears, email <ilarj@nas.edu>.

Westerfield, M. (2000). ***The Zebrafish Book. A Guide for the Laboratory Use of Zebrafish (Danio Rerio).*** 4th ed., Univ. of Oregon Press, Eugene.
URL: http://zfin.org/zf_info/zfbook/zfbk.html
Descriptors: zebrafish, *Danio rerio*, developmental genetics, animal care, methodology, breeding, histology, homozygosity, pattern formation
Note: Hardcopies of the 4th edition of The Zebrafish Book can be obtained for a nominal fee from the Zebrafish International Resource Center, 5274 University of Oregon, Eugene, OR 97403 USA; fax: 541-346-6151.

Winn RN (2001) **Transgenic fish as models in environmental toxicology.** Fish Models in Biomedical Research. *ILAR Journal* 42(4):
NAL Call No. QL55 A1I43
URL: http://dels.nas.edu/ilar/jour_online/42_4/transg.asp
Historically, fish have played significant roles in assessing potential risks associated with exposure to chemical contamination in aquatic environments. Considering the contributions of transgenic rodent models to biomedicine, it is reasoned that the development of transgenic fish could enhance the role of fish in environmental toxicology. Application of transgenic fish in environmental studies remains at an early stage, but recent introduction of new models and methods demonstrates progress. Rapid advances are most evident in the area of in vivo mutagenesis using fish carrying transgenes that serve as recoverable mutational targets. These models highlight many advantages afforded by fish as models and illustrate important issues that apply broadly to transgenic fish in environmental toxicology. Development of fish models carrying identical transgenes to those found in rodents is beneficial and has revealed that numerous aspects of in vivo mutagenesis are similar between the two classes of vertebrates. Researchers have revealed that fish exhibit frequencies of spontaneous mutations similar to rodents and respond to mutagen exposure consistent with known mutagenic mechanisms. Results have demonstrated the feasibility of in vivo mutation analyses using transgenic fish and have illustrated their potential value as a comparative animal model. Challenges to development and application of transgenic fish relate to the needs for improved efficiencies in transgenic technology and in aspects of fish husbandry and use. By taking advantage of the valuable and unique attributes of fish as test organisms, it is anticipated that transgenic fish will make significant contributions to studies of environmentally induced diseases.
Descriptors: fish models, human disease, biomedical research, *Fundulus*, lambda, medaka, plasmid, transgenic
Note: Abstract reprinted with permission from ILAR Journal, Institute for Laboratory Animal Research, National Academy of Sciences, 500 Fifth Street, NW, Washington, DC 20001 (http://dels.nas.edu/ilar). To purchase copies of this article and the entire issue in which it appears, email <ilarj@nas.edu>.

Woolaston RR (1998) **Breeding livestock for reduced reliance on chemicals.** *Proceedings of the 6th World Congress on Genetics Applied to Livestock Production, Armidale, Australia, January 11-16, 1998. Volume 27: Reproduction; fish breeding; genetics and the environment; genetics in agricultural systems; disease resistance; animal welfare; computing and information technology; tree breeding.* World Congress on Genetics Applied to Livestock Production. Armidale, Australia. 145-152
NAL Call No. SF105 W67 1998
This paper begins with a brief discussion of why chemicals are used in animal production and then considers the problems associated with their use, including residues in animal products, resistance of bacteria and parasites, environmental consequences and carcass damage. It is argued that many traits targeted by chemicals are heritable and, therefore, breeding can achieve similar ends, although it would be more practicable, in most cases, to use both methods to achieve improvement in productivity. Disease is an example of a trait where reliance on veterinary chemicals can be reduced by breeding for resistance. The optimal emphasis that should be placed on disease resistance in breeding programmes depends on a number of factors, some of which are difficult to measure. Genetic correlations between productivity and disease resistance appear to be unfavourable or neutral in a number of species.
Descriptors: breeding, disease resistance, livestock, diseases

Web Resources:

Aquaria Fish Models of Human Disease
http://www.xiphophorus.org/100300s1/css/100300s1_1.htm

Aquatic Animal Models of Human Disease Conference. September 29-October 2, 2003. Manassas, VA.
http://pasteur.atcc.org/aqua/AbstractSubmission.cfm

Marine Biotechnology Volume 3 – Supplement (2001) Special Issue: Aquaria Fish Models of Human Disease.
http://www.springerlink.com/app/home/issue.asp?wasp=3n2ltwyyvk013y2gnt2l&referrer=parent&backto=journal,8,26;linkingpublicationresults,1,1

How the *Xiphophorus* Problem Arrived in San Marcos, Texas
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,1,30;journal,8,26;linkingpublicationresults,1,1

Cell Cultures and Retroviral Particles From a Tumor of a Moray Eel
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,2,30;journal,8,26;linkingpublicationresults,1,1

***Xiphophorus* Genetic Linkage Map: Beginnings of Comparative Gene Mapping in Fishes**
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,3,30;journal,8,26;linkingpublicationresults,1,1

Four Resource Centers for Fishes: Specifies, Stocks, and Services
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,4,30;journal,8,26;linkingpublicationresults,1,1

Cryopreservation in Aquarium Fishes
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,5,30;journal,8,26;linkingpublicationresults,1,1

Progression of Infection and Tumor Development in Damselfish
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,6,30;journal,8,26;linkingpublicationresults,1,1

Resolution of UV-Induced DNA Damage in *Xiphophorus* Fishes
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,7,30;journal,8,26;linkingpublicationresults,1,1

Use of the Japanese Pufferfish (*Fugu rubripes*) in Comparative Genomics
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,8,30;journal,8,26;linkingpublicationresults,1,1

Bacteriophage 5 and Plasmid pUR288 Transgenic Fish Models for Detecting In Vivo Mutations
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,9,30;journal,8,26;linkingpublicationresults,1,1

Transformation-Associated Recombination (TAR) Cloning of Tumor-Inducing Xmrk2 Gene from *Xiphophorus maculates*
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,10,30;journal,8,26;linkingpublicationresults,1,1

Production of Transgenic Live-Bearing Fish and Crustaceans with Replication-Defective Pantropic Retroviral Vectors
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,11,30;journal,8,26;linkingpublicationresults,1,1

Application of Fluorescence In Situ Hybridization (FISH) to Fish Genetics and Genome Mapping
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,12,30;journal,8,26;linkingpublicationresults,1,1

Comparative Genomics of Medaka: The Major Histocompatibility Complex (MHC)
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,13,30;journal,8,26;linkingpublicationresults,1,1

The Medaka as a Model for Studying Germ-Cell Mutagenesis and Genomic Instability
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,14,30;journal,8,26;linkingpublicationresults,1,1

Retinoblastoma Gene Mutations in Chemically Induced Liver Tumor Samples of Japanese medaka (*Oryzias latipes*).
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,15,30;journal,8,26;linkingpublicationresults,1,1

Introduction: Aquaria Fish Models of Human Disease
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,16,30;journal,8,26;linkingpublicationresults,1,1

Genetic Analysis of Neoplasia Induced by N-Nitroso-N-methylurea in *Xiphophorus* Hybrid Fish
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,17,30;journal,8,26;linkingpublicationresults,1,1

A Proposed Classification Scheme for *Xiphophorus* Melanomas Based on Histopathologic Analyses
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,18,30;journal,8,26;linkingpublicationresults,1,1

Lesser Known Aquarium Fish Tumor Models
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,19,30;journal,8,26;linkingpublicationresults,1,1

In Appreciation of Klaus D. Kallman
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,20,30;journal,8,26;linkingpublicationresults,1,1

Genetic Analysis of Susceptibility to Spontaneous and UV-Induced Carcinogenesis in *Xiphophorus* Hybrid Fish
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,21,30;journal,8,26;linkingpublicationresults,1,1

Aquaria Fish Models Attendee Roster September 21-24, 2000
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Genetic Relationship of Tumor-Associated Piscine Retroviruses
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,23,30;journal,8,26;linkingpublicationresults,1,1

Genomic Plasticity and Melanoma Formation in the Fish *Xiphophorus*
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,24,30;journal,8,26;linkingpublicationresults,1,1

Dioxin Toxicology and the Aryl Hydrocarbon Receptor: Insights from Fish and Other Non-traditional Models
http://www.springerlink.com/app/home/contribution.asp?wasp=5n5d6ynmpg1kwkda7y47&referrer=parent&backto=issue,25,30;journal,8,26;linkingpublicationresults,1,1

Relative Base Excision Repair in *Xiphophorus* Fish Tissue Extracts
http://www.springerlink.com/app/home/contribution.asp?wasp=p3d6f5hcwmcrqmfdmeqm&referrer=parent&backto=issue,26,30;journal,8,26;linkingpublicationresults,1,1

Aquaria Fish Models of Human Disease: Reports and Recommendations from the Working Groups
http://www.springerlink.com/app/home/contribution.asp?wasp=p3d6f5hcwmcrqmfdmeqm&referrer=parent&backto=issue,27,30;journal,8,26;linkingpublicationresults,1,1

Three Unique Experimental Fish Stories: Poecilia (the Past), *Xiphophorus* (the Present), and Medaka (the Future)
http://www.springerlink.com/app/home/contribution.asp?wasp=p3d6f5hcwmcrqmfdmeqm&referrer=parent&backto=issue,28,30;journal,8,26;linkingpublicationresults,1,1

Reporter Gene Expression in Fish Following Cutaneous Infection with Pantropic Retroviral Vectors
http://www.springerlink.com/app/home/contribution.asp?wasp=p3d6f5hcwmcrqmfdmeqm&referrer=parent&backto=issue,29,30;journal,8,26;linkingpublicationresults,1,1

Utility of Natural Populations for Microarray Analyses: Isolation of Genes Necessary for Functional Genomic Studies
http://www.springerlink.com/app/home/contribution.asp?wasp=p3d6f5hcwmcrqmfdmeqm&referrer=parent&backto=issue,30,30;journal,8,26;linkingpublicationresults,1,1

Kent ML, Spitsbergen JM, Matthews JM, Fournie JW, Westerfield (2002) **Diseases of Zebrafish in Research Facilities.**
http://zfin.org/zf_info/stckctr/dis_man/Fish_Diseases.html

UM MFBSC Marine Models
http://www.rsmas.miami.edu/groups/niehs/science/models.jsp

> Marine Models of Human Disease. ... Importantly, fish and invertebrates represent a vast phylogenetic diversity that far exceeds that of mammals. ...

Model Organisms: Fish
http://www.wellcome.ac.uk/en/genome/genesandbody/hg05b004.html

The Zebrafish Information Network
http://zfin.org/cgi-bin/webdriver?MIval=aa-ZDB_home.apg

Toxicological Pathology Atlas of Small Laboratory Fish
part I - normal histology and effects of endocrine disruptors in zebrafish *Danio rerio*
Leo van der Ven and Piet Wester
http://arch.rivm.nl/milieu/rivmzfatlas/fishtoxpat/index_uk.html

Westerfield, M. (2000). **The zebrafish book. A guide for the laboratory use of zebrafish (*Danio rerio*).** 4th ed., Univ. of Oregon Press, Eugene.
http://zfin.org/zf_info/zfbook/zfbk.html

> Descriptors: zebrafish, *Danio rerio*, developmental genetics, animal care, methodology, breeding, histology, homozygosity, pattern formation
> Note: Hardcopies of the 4th edition of The Zebrafish Book can be obtained for a nominal fee from the Zebrafish International Resource Center, 5274 University of Oregon, Eugene, OR 97403 USA; fax: 541-346-6151.

3.2.6. SELECTED HUSBANDRY TOPICS

3.2.6.1. Domestication of Stocks

Ambali A (1997) **The Relationship Between Domestication and Genetic Diversity of Oreochromis Species in Malawi: *Oreochromis shiranus* shiranus (Boulener) and *Oreochromis shiranus* chilwae (Trewavas).** *Dissertation Abstracts International Part B: Science and Engineering.* 58(4):1655

NAL Call No. Film S-1806

The fish species domesticated in most African aquaculture activities have not been genetically identified and characterized; and the distribution of their genetic diversity is not known. As a result there is continuous mixing of otherwise genetically differentiated strains leading to loss of between-population genetic variation. In this study, microsatellite DNA markers for tilapia were developed to analyze genetic diversity of wild and domesticated populations of *O. shiranus* species in Malawi. The primers developed with *O. shiranus* DNA amplified microsatellites in other species of the tilapiine fishes. Genetic relationships among populations of *O. shiranus* in Malawi were determined and it was observed that the populations in Lakes Chilwa and Chiuta belonged to *O. shiranus chilwae* while the population in Lake Malombe belonged to *O. shiranus shiranus*. Measures of genetic diversity declined in the domesticated populations compared to wild populations. The loss of diversity was correlated with the time elapsed since the founding of farm stock and population genetic differentiation was also strongly influenced by the pattern of known exchange of germplasm among farms. Social factors, as measured by transfer proximity, are the key determinants of genetic distance, not geographic distance. Genetic diversity in the reservoir populations declined with increase in predator populations which were stocked either artificially to control tilapia populations, or naturally through streams. Uncontrolled transfer of germplasm between reservoirs resulted in genetic contamination of the populations. Socio- economic analysis of small scale farms in Malawi showed that growout operations were economically viable primarily because pond inputs comprised recycled on- farm and household waste. The predominance of integrated crop/livestock/fish farming system was a favorable indicator of the possibility of involving farmers in community-based aquaculture biodiversity conservation programs at farm level. The programs would benefit from the knowledge and experience farmers had already acquired in conserving indigenous breeds of livestock and crops.

Descriptors: phylogenetics, fish culture, freshwater aquaculture, genetics, biopolymorphism, *Oreochromis shiranus chilwae*, *Oreochromis shiranus shiranus*, Africa

Ambali AJD (1998) **Domestication selection of indigenous tilapia species for aquaculture in the Malawian aquaculture industry.** *African Fishes and Fisheries Diversity and Utilisation. Poissons et Peches Africains Diversite et Utilisation.* p. 190

Domestication selection and testing of aquaculture species has been based mainly on growth rate of the species and its ability to breed in culture systems. There is limited effort to explore the genotype-environmental interaction since most of the research is centralised in few government stations. In this study it has been observed that most domesticated populations

have been established from small effective populations to the effect that the genetic diversity of the populations has been lost, for instance in Malawi wild populations of *O. shiranus spp.* have higher mean microsatellite DNA allele number than domesticated populations, 12.0 ± 4.2 and 3.5 ± 1.0, respectively. Findings in this research programme suggest that a genetic species selection and testing procedure for indigenous tilapias based on collimation, scale morphology and brookstock management enriches the already existing domestication protocols which mainly screen for performance of species subjected to various feeding and water quality regimes, and preserves genetic diversity in cultured populations.
Descriptors: aquaculture development, endemic species, aquaculture, fish culture, domestic species, *Oreochromis shiranus*, Malawi
ASFA; Copyright © 2003, FAO

Ambali AJD (1997) **Genetic considerations in the domestication of indigenous species for aquaculture: Theory and practice.** Report of the Technical Consultation on Species for Small Reservoir Fisheries and Aquaculture in Southern Africa. Livingstone, Zambia, 7-11 November 1994. *Algorithms and Complexity Report.* No. 19:22-23
Domesticated stocks have been subjected to processes of evolution as humans impose selection pressures on crops and animals in order to adapt to the environmental conditions and human preferences. Aquaculture is relatively young and most cultured fish species are still close to their wild counterparts. Most South African countries have rich genetic resources of fishes in the natural waters. Genetic characterization is an essential component of the screening package for the candidate indigenous species for aquaculture in order to determine the amount of genetic variation that exists and identify the various strains of a particular species. Most of the domestication programmes for fish in Africa have been constrained by the fact that they work on species that have not been properly identified. Information on population structure of a species is important for determining the genetic differentiation of wild populations. Hybridization can be a genetic improvement approach to obtain heterosis of some fitness traits. If hybridization is chosen as an approach for improving the performance of indigenous populations then there should be well established genetic characterization records in order to monitor the long-term purity of the broodstock. The most important consideration during genetic material collection and management of the populations in stations is to conserve the gene pool to prevent genetic drift and detrimental levels of inbreeding in order to minimize changes over time in the gene and genotype frequencies. The selection and testing of indigenous species is considered briefly.
Descriptors: fish culture, endemic species, genetics, hybridization, aquaculture development, Africa, Southern
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Ambali AJD, Doyle RW, Cook DI (1999) **Genetic changes in *Oreochromis shiranus* (Trewavas) associated with the early stages of national aquaculture development in Malawi.** *Aquaculture Research.* 30(8):579-588
NAL Call No. SH1 F8
A study was carried out to investigate the genetic diversity during domestication of *Oreochromis shiranus* (Trewavas) and to see if it could be associated with events in the known history of aquaculture development in Malawi. Five polymorphic microsatellite loci were scored in 14 populations of *O. shiranus* and one population of *O. mossambicus* (Peters). The mean number of alleles per locus ranged from 4.4 ± 1.03 to 13.2 ± 3.31 and was higher in the wild populations than in the domesticated populations. Other measures of genetic diversity were also lower in the domesticated compared with the wild populations, and the

decline in diversity was correlated with the time elapsed since the founding of the farm stocks. Ordination analysis grouped domesticated populations into three: (1) those that trace their genealogy from Lakes Chiuta and Chilwa populations and are now spread all over the country; (2) those that come from Lakes Malawi and Malombe; and (3) hybrids between *O. shiranus* and *O. mossambicus*. Genetic differentiation among farms was strongly influenced by the pattern of known exchanges among the farmers and introgressive hybridization that had occurred between *O. shiranus* and *O. mossambicus* in the farmers' ponds. Thus, the process of genetic changes in the species subsequent to domestication are best explained and predicted by socio-economic factors that influence the behaviour of farmers, rather than by the time-and-distance models of standard population genetics.
Descriptors: man-induced effects, aquaculture techniques, brood stocks, fish culture, population genetics, aquaculture development, microsatellites, genetic diversity, Malawi, *Oreochromis shiranus*, *Oreochromis mossambicus*

Brummett RE (no date given) **Research on improved germplasm for aquaculture at the ICLARM Regional Resarch Center for Africa and West Asia and a note on the domestication of *Heterotis niloticus*.** *Biodiversity and sustainable use of fish in the coastal zone. ICLARM Conference Proceedings* 63:56-58
ISSN: 0115-4435
This paper highlights the ICLARM's genetic research programme at the Regional Research Centre for Africa and West Asia in Abbassa, Egypt which began in 1998, and the developments of this programme. Under this programme populations of the species *Oreachromis niloticus*, *O. aureus*, *Sarotherodon galilaeus* and *Tilapia zillii* were characterized in terms of growth, yield and food conversion efficiency. Besides, the objectives of the new collaborative project entitled "Genetic Enhancement of Tropical Aquaculture species by Combined Selection Marker-Assisted Selection and Quantitative Trail Loci (QTL) Mapping" are briefed. This project is proposed to have four phases (1) measuring the heritability and genetic correlations for growth, (2) gene mapping of the QTL for these straits, (3) development of selection indices and (4) evaluation of selection. Other activities of ICLARM lake seeking funding for a network that would bring together the main research centres working on Heterotis niloticus to coordinate rationalize and share funding.
Descriptors: marine fish, fish culture, aquaculture techniques, genetics, heterotis niloticus, ASE, Africa, research programmes, domestication, biodiversity, germplasm

Chiyokubo T, Shikano T, Nakajima M, Fujio Y (1998) **Genetic features of salinity tolerance in wild and domestic guppies (*Poecilia reticulata*).** *Aquaculture*. 167(3-4):339-348

To elucidate the genetic features of salinity tolerance in wild and domestic guppies, *Poecilia reticulata*, the present study examined the salinity tolerance in four wild populations and 13 domestic strains. Salinity tolerance was measured as survival time after transfer from fresh water to 35 ppt seawater. In the wild guppies, all four of the wild populations showed significantly higher salinity tolerance than the 13 domestic strains. After domestication of the wild guppies, their salinity tolerance significantly decreased with reductions in salinity tolerant individuals, suggesting inbreeding depression. In the domestic guppies, on the other hand, strain differences were observed during both 1993 and 1997. A significant positive correlation between those in 1993 and 1997 suggests that the genetic constitutions for salinity tolerance have stabilized in each strain as a consequence of long-term maintenance. F_1

hybrids between the domestic strains showed significantly higher salinity tolerance with many salinity tolerant individuals which were not observed in their parental strains, thus indicating a heterotic effect. The salinity tolerance in the F_1 hybrids reached the same level as that in the wild populations. Salinity tolerance significantly decreased with reductions in the salinity tolerant individuals in the F_2.The results of the domestications and the cross experiments suggested that the significant difference in salinity tolerance between the wild and the domestic guppies was caused by heterosis and inbreeding depression.
Descriptors: freshwater fish, hybrids, genetics, natural populations, salinity tolerance, fish culture, population genetics, hybrid culture, inbreeding depression, population studies, *Poecilia reticulata*, domestication, inbreeding, heterosis, guppy, millions fish, rainbowfish
ASFA; Copyright © 2003, FAO

Fang Yongqiang, Weng Youzhu, Yang Yao, Chen Lan (1998) **Introductive domestication and culture of turbot in Xiamen.** *Journal of oceanography in Taiwan Strait/Taiwan Haixia. Xiamen.* 20(3):356-362
ISSN: 1000-8160
The paper reported the experiment on introductive domestication and culture of two batches of turbot which were from Weihai, Shandong Province in 1997 and 1999 respectively, with whole length about 3 cm to 5 cm. The two batches fry were cultured indoors. The experimental results indicated that turbot had a fast growth speed, and the average body weight in eleven-month-old turbot can reach to 797 g (smallest: 350 g, largest: 1,250 g), and turbot can safely spend summer indoors. The experiment also found that Ziqi may be as a growth promote for turbot. In addition, the turbot fry with whole length about 6 cm to 10 cm and body weight 10-30 g were cultured for five months in the net cages of seawater, the average body weight can reach to about 400 g. these results will provide scientific base for the turbot culture in factories and net cages.
Descriptors: fish culture, domestication, introduced species, tests, disease control, *Scophthalmus maximus*
ASFA; Copyright © 2003, FAO

Fernoe A, Jaervi T (1998) **Domestication genetically alters the anti-predator behaviour of anadromous brown trout (*Salmo trutta*) - a dummy predator experiment.** *Nordic Journal of Freshwater Research.* 74:95-100
NAL Call No. SH287.N6
Domesticated, anadromous brown trout juveniles have been reported to be more prone to take risks in order to obtain food than juveniles from a wild strain. This study looked at changes in the actual response to an attacking predator of juvenile sea trout due to domestication. The progeny of wild and sea-ranched sea trout were reared in hatcheries under identical conditions for one year. When exposed to a model predator, domesticated juveniles were more likely to swim or sink to the bottom and to keep still (freeze) than wild juveniles, which more often escaped by panic swimming. No difference was found in escape distance. The results support the view that the hatchery environment selects for risk-taking individuals.
Descriptors: culture effects, cultured organisms, natural populations, protective behaviour, predation, genetics, predator prey interactions, hatcheries, fish culture, domestication, *Salmo trutta*, brown trout
ASFA; Copyright © 2003, FAO

Gadagkar SR (1998) **Social Behaviour and Growth Rate Variation in Cultivated Tilapia (*Oreochromis niloticus*).** *Dissertation Abstracts International Part B: Science and Engineering.* 59(2):no page numbers given

NAL Call No. Film S-1806

This study was undertaken to understand the behavioural causes of growth variation in cultivated fish and to study the genetics of agonistic behaviour vis-a-vis growth rate. The fish studied was a laboratory population of the Nile tilapia, *Oreochromis niloticus*, being maintained at the Marine Gene Probe Laboratory, Dalhousie University. Ten maternal half-sib families were produced by fertilizing the eggs of each female with the sperm from four different males. The fish within each half-sib family were all pooled together for the behaviour as well as growth experiments, in order to expose each fish to the full range of microenvironments within the half-sib family, and to eliminate replicate variance. Upon termination of the experiments, the male parent of each fish was determined using DNA fingerprinting using microsatellites. the behavioural observations were made by randomly pairing fish from within each half-sib pool, soon after swim-up, and counting the number of aggressive and submissive behaviours displayed by each member of the pair. Two derived variables (net aggression, viz. aggression minus submission; and total agonistic activity, viz. aggression plus submission) were also constructed for each fish. Growth experiments were conducted by rearing fish from the same 10 half-sib groups in each of two types of competitive environments, high interaction (HI), and low interaction (LI).

Descriptors: social behaviour, aggressive behaviour, domestication, fish culture, *Oreochromis niloticus*

Gjedrem T (2000) **Genetic improvement of cold-water fish species.** *Aquaculture Research.* 31(1):25-33

NAL Call No. SH1 F8

Carnivorous fish are two to three times as efficient as pigs and broilers in converting energy and protein to edible food for humans. As the domestication of fish continues, they will become more and more efficient and competitive with these industries. In the future, this will be very important, as more efficient utilization of available food resources is required to supply the growing human population with enough food. Today, about 1% of aquaculture production is based on genetically improved fish and shellfish. For salmonid fishes, we have the necessary knowledge to establish efficient breeding programmes. Large genetic variation has been associated with important economic traits. For growth rate, heritability ranges from 0.2 to 0.3, with a coefficient of variation of 20-30%. This implies that it is possible to obtain large responses from selection for growth rate. In several large-scale experiments and in breeding programmes, 10-15% genetic change has been obtained per generation, which is much higher than that reported for other farm animals. In Norway, extensive breeding experiments with Atlantic salmon and rainbow trout were started in 1971. For the first two generations of selection, the breeding goal was growth rate. Age at sexual maturation (measured as frequency of grilse) was then included. From the fifth generation, disease resistance (measured by challenge test for furunculosis and the virus ISA) and meat quality (measured as fat percentage, fat distribution and flesh colour) were included. Today, Norsk Lakseavl AS (Norwegian Salmon Breeding Company Ltd) or NLA runs the National Breeding Programme and has two breeding stations where 400 full-sib and half-sib families of Atlantic salmon are tested in each of four year classes. For rainbow trout, the number of families totals 120 in each of three year classes. In 1997, the Norwegian production was 310 000 tons of Atlantic salmon and 33 000 tons of rainbow trout. At present, about 65% of the

salmon and trout produced in Norway use genetically improved fish from NLA and multipliers. The cost-benefit ratio for the National Breeding Programme in Norway is estimated to be 1:15.
Descriptors: fish culture, selective breeding, growth curves, biological age, disease resistance, body conditions, chemical composition, sexual maturity, aquaculture techniques, yield, aquaculture, breeding, heritability, genetic diversity, *Salmo salar*, *Oncorhynchus mykiss*, Salmonidae, Norway, rainbow trout, Atlantic salmon, salmonids

Harada Y, Yokota M, Iizuka M (1998) **Genetic risk of domestication in artificial fish stocking and its possible reduction.** *Researches on Population Ecology.* 40(3):311-324
NAL Call No. 420 K99
Genetic hazards associated with the stocking of fish juveniles produced in hatcheries were studied with simple mathematical models. Domestication is the process of acquiring a genetic characteristics that are advantageous in a hatchery environment but that are disadvantageous in a natural environment due to the selection pressure in the hatchery differing from that in the natural environment. Conditions for the propagation of mutants enhancing domestication were obtained for a variety of stocking strategies specified by parameters related to hatchery productivity and kind of brood stock used. By using this, the possibility of reducing the risk of domestication was studied. As a means of reducing the risk, selective use of wild-born individuals for brood stock was considered. The effectiveness of this was analyzed for both the cases where all brood stock is collected from the wild, and the male brood stock is collected from the wild and the female brood stock is born and reared in a hatchery. We also estimate how much hatchery release can be increased without increasing the risk by employing these means. It is concluded that the use of only male brood stock from the wild is not very effective in reducing the risk of domestication. Further, it is concluded that selective use of the wild-born individuals of both sexes for brood stock is highly desirable if the contribution of released individuals to the natural reproduction is high. In other words, substantial increase of hatchery release may be possible while keeping risk at a level comparable to that under moderate hatchery release, if it is accompanied by the selective use of wild-born individuals for brood stock.
Descriptors: domestication, genetic factors, risks, stocking, wildlife management, brood stocks, genetics, hatcheries, stocking (organisms), fish culture, Pisces

Hassin S, de Monbrison D, Hanin Y, Elizur A, Zohar Y, Popper DM (1997) **Domestication of the white grouper, *Epinephelus aeneus* 1. Growth and reproduction.** *Aquaculture* 156(3-4):309-320
NAL Call No. SH1 A6
The growth and reproductive biology of the white grouper, *Epinephelus aeneus*, were studied in captive fish to determine its potential for aquaculture. About 250 fish were captured by fishermen along the Mediterranean coast and maintained in 16 m3 concrete tanks supplied with sea water in a flow-through system. The captive fish fed readily on dry pellets supplemented with chopped frozen fish and gained an average of 3.3 g/day during the initial growth phase (0.5-1.5 kg), and 11.3 g/day during the secondary growth phase (1.5-3.0 kg). Using ovarian biopsies, the sexual development of 17 females was closely monitored for 3 years, and for 1 year in an additional 47 females. In adult females held in captivity, the oocytes reached the final stages of vitellogenesis, however, final oocyte maturation, ovulation and spawning did not occur. Sustained release of [d-Ala6,Pro9NEt]-GnRH from

implanted devices was highly effective in inducing ovulation, but did not result in natural spawning. Repeated implantations resulted in 2-3 ovulations per reproductive season, which lasted from April through September. The ovulated females were manually stripped and the eggs were artificially fertilized, resulting in millions of fertilized eggs and larvae. The average fecundity per female was 242 343 eggs (kg BW)-1yr-1. In some of the young females, early vitellogenesis did not lead to the final stages of vitellogenesis. Instead, the vitellogenic oocytes underwent rapid atresia. Monitoring individual fish demonstrated that E. aeneus is a protogynous hermaphrodite, changing sex from female to male, confirming reports by other authors. Sex inversion occurred both spontaneously and after implantation with 17 alpha -methyltestosterone. The rapid growth rates and the potential for induced spawning in captivity make the white grouper an excellent candidate for mariculture.
Descriptors: commercial species, fish culture, marine fish, aquaculture development, induced breeding, fish eggs, *Epinephelus aeneus*, white grouper

Hollebecq MG, Haffray P (1999) **Genetic improvements.** *Carp: biology and culture.* pp. 101-123.
NAL Call No. SH167 C3 C3713 1999
Chapter 4 focuses on genetic improvement: domestication; starins identification and management, performance assessment, breeding and hybridizations; selection methods; sex control and polyploidization; molecular genetics and transgenesis
Descriptors: fish culture, population genetics, selective breeding, brood stocks, Cyprinidae, *Cyprinus carpio*

Jacobs JM, Lindell S, Van Heukelem W, Hallerman EM, Harrell RM (1999) **Strain evaluation of striped bass (*Morone saxatilis*) under controlled conditions.** *Aquaculture.* 173(1-4):171-177
NAL Call No. SH1 A6
Commercial hybrid striped bass production is one of the fastest growing segments of the US aquaculture industry. However, broodstock domestication and selective breeding on a production scale have yet to be exploited. We reared progeny from five wild striped bass (*Morone saxatilis*) populations representing New York to Florida at two facilities (Horn Point Laboratory (HPL), Cambridge, MD and AquaFuture (AFI), Turners Falls, MA). Some 19 families were grown for approximately 150 days in recirculating aquaculture systems and evaluated for differences in growth rate. Maryland and Florida Blackwater populations exhibited higher growth rates than the South Carolina and New York strains ($P<.05$) with Florida St. John's fish showing intermediate growth. All strains grew significantly faster at AFI than HPL ($P=0.001$) with absolute growth rate (g/day) strongly correlated at the two facilities ($SCC=0.823$, $P=0.0001$).
Descriptors: fish culture, brood stocks, hybrid culture, phylogenetics, recirculating systems, *Morone saxatilis*, rockfish

Lal KK, Ponniah AG (2000) **Reproductive biology estimators for conservation and culture of fish.** *Endemic fish diversity of Western Ghats NBFGR-NATP Publication.* 1: 289-292
Captive breeding is an essential prerequisite for successful domestication of a new species; it also plays a significant role in conservation of wild germplasm through maintaining live gene banks of captive brood stocks and for repopulating depleted natural habitats. Different reproductive biology estimators are useful for predicting reproductive success as well as in

developing manipulative techniques for both culture and conservation. An examination is made of various estimators which can be used individually and collectively depending upon the end objective. The following parameters are discussed: reproductive strategy; sexual dimorphism; sex ratio; age and size at first maturity; potential fecundity; gonad features; gonadosomatic index; and, oocyte diameter.
Descriptors: freshwater fish, fish culture, reproduction, resource conservation, sexual maturity, spawning populations, India

Lepage O, Oeverli Oe, Petersson E, Jaervi T, Winberg S (2000) **Differential stress coping in wild and domesticated sea trout.** *Brain, Behavior and Evolution.* 56(5):259-268
ISSN: 0006-8977
Offspring of wild and sea-ranched (domesticated) sea trout (*Salmo trutta*) originating from the same river, were reared under identical hatchery conditions from the time of fertilization. At one year of age individual fish were exposed to two standardized stressors; transfer to a novel environment, with or without a simultaneous predator exposure. Blood plasma concentrations of glucose and cortisol were analyzed along with brain levels of dopamine (DA), 3,4-hydroxyphenylacetic acid (DOPAC, a major DA metabolite), serotonin (5-hydroxytryptamine, 5-HT), and 5-hydroxyindoleacetic acid (5 HIAA, a major 5-HT metabolite). Transfer to a novel environment, alone as well as in combination with predator exposure, resulted in elevated plasma concentrations of glucose and cortisol. Moreover, exposure to these stressors resulted in elevated brain levels of 5-HT and 5-HIAA, as well as elevated brain 5-HIAA/5-HT and DOPAC/DA ratios. Wild trout displayed significantly higher post stress plasma glucose levels than domesticated fish. Similarly, following stress, brain 5-HIAA/5-HT and DOPAC/DA ratios were significantly higher in wild than in domesticated fish. These differences were not caused by differences in brain levels of 5-HIAA and DOPAC, but instead by differences in brain 5-HT and DA concentrations. These results suggest that domestication results in attenuated stress responses in trout, and that alterations in brain monoamine neurotransmission are part of this effect.
Descriptors: stress, adaptability, novelty, predation, glucose, hydrocortisone, domestication, neurotransmitters, biological stress, fish physiology, stocking (organisms), cultured organisms, natural populations, *Salmo trutta*, brown trout

Liao IC (2000) **The state of finfish diversification in Asian aquaculture.** Recent advances in Mediterranean aquaculture finfish species diversification. Proceedings of the Seminar of the CIHEAM Network on Technology of Aquaculture in the Mediterranean (TECAM), jointly organized by CIHEAM and FAO, Zaragoza (Spain), 24-28 May 1999. *Cahiers Options Mediterraneennes.* Zaragoza 47:109-125
ISSN: 1022-1379
Aquaculture in Asia has a rich and experience-filled history of more than 2500 years. Asia is recognized as the leading aquaculture region in the world contributing 90% of the total world aquaculture production in 1996. About 100 species of finfish listed in the FAO yearbook are cultured in this area. This diversity of cultured finfish may be attributed to environmental and social factors. Recently, economic prosperity allowed people to change their preferences on seafood consumption. Furthermore, aquarium enthusiasts have got the opportunity to keep ornamental fishes as a delightful hobby. These factors motivated aquafarmers to diversify their cultured species while the aquarists imported more exotic species. The exploitation of new cultured species and introduction of exotic species are the two means in diversification.

Generally, freshwater finfish are the primary exotic species in most countries of Asia. However, owing to their high economic value and market demand, marine finfish and ornamental fish have played the principal role to diversification. Applications of biotechnology in aquaculture and domestication are other possible approaches that may yield new species for culture. Species diversification offers both biological and economic benefits, and is thus worth to pursue in the long-term. The approaches to finfish cultured diversification in Asia may provide a good example for other areas to follow.
Descriptors: aquaculture, aquaculture development, induced breeding, domestication, introduced species, check lists, hybridization, fish culture, species diversity, Taiwan

Mamontov YuP, Rekubratskiy AV (1998) **Carp domestication and breeding.** *Rybovodstvo i Rybolovstvo. Moscow.* 3-4:31-33 (In Russian with English summary)
The history of domestication of wild carp as an ancestor of carps (*Cyprinidae*) in Asia and Europe as well as their morphological differences are considered. The next signs of domestication are discussed: 1) specimens have economical value; 2) the breeding is under man's control; 3) carps behaviour differs from its wild ancestor; 4) some hatchery-bred fish are not able to survive without man's help.
Descriptors: fish culture, breeding ponds, domestication, I, China, People's Rep., Japan, Europe, Inland Waters, Israel

Sola L, De Innocentis S, Rossi AR, Crosetti D, Scardi M, Boglione C, Cataudella S (1998) **Genetic variability and fingerling quality in wild and reared stocks of European sea bass, Dicentrarchus labrax. Genetics and breeding of Mediterranean aquaculture species.** *Cahiers Options Mediterraneennes.* Zaragoza. 34:273-280
ISSN: 1022-1379
The development of sustainable aquaculture models requires an increasing knowledge of hatchery production practices. This preliminary study has aimed at investigating the domestication process in the European sea bass, *Dicentrarchus labrax.* Genetic variability and frequencies of anatomical abnormalities were inspected in 5 hatcheries and in one sample of wild sea bass juveniles. Gene-enzyme analysis (carried out through starch gel electrophoresis on about 300 individuals) revealed low genetic distances among groups, and allelic and genotypic frequency shifts in the hatchery groups when compared to the wild one. The analysis of differences in meristic counts and physical anomaly types and frequencies (evaluated on more than 430 juveniles) revealed a wide morphological variation among the hatchery groups and also between these and the wild group, with some hatchery-specific trends.
Descriptors: fish culture, fingerlings, genetics, biopolymorphism, stocks, *Dicentrarchus labrax*, MED, Europe, comparative studies

Web Resources:

An Illustration of Domestication Selection In a Hatchery Program of Steelhead.
http://www-heb.pac.dfo-mpo.gc.ca/congress/2002/Hatchery/Reisenbichler.pdf

Analyzing Genetic and Behavioral Changes During Salmonid Domestication
http://www.cbfwa.org/files/awp00/projects/20045.htm

Aspects of Animal Welfare and Aquaculture -A Compendium of Selected Literature by Richard D. Moccia and Kristopher P. Chandroo; Aquaculture Centre, University of Guelph, Guelph, Ontario, Canada
http://www.aps.uoguelph.ca/~aquacentre/aec/publications/welfare-bib.html

Devlin RH Biagi CA, Yesaki TY, Smailus DE, Byatt DC (2001) **Growth of domesticated transgenic fish: A growth-hormone transgene boosts the size of wild but not domesticated trout.** *Nature* 409:781-782
http://www.nature.com/cgi-taf/DynaPage.taf?file=/nature/journal/v409/n6822/full/409781a0_r.html

3.2.6.2. Harvest & Slaughter

Akse L, Midling K (2000) **Slaughtering of Atlantic Halibut (*Hippoglossus hippoglossus*): Effect on Quality and Storing Capacity.** In: *Farmed Fish Quality.* Eds: Kestin SC, Warris PD. Blackwell Science Inc. 448 pp.
NAL Call No. SH151 F37 2001
Through 15 years of intensive biological research, Atlantic halibut is now established as the most promising species in Norwegian marine aquaculture. Annual production is still small, but is expected to grow rapidly. However, knowledge on muscle quality and what factors affect the product in this species are scarce. In this project a number of experiments were conducted on farmed Atlantic halibut. Except for the initial pH, we were not able to detect major differences in post-mortem quality between the stressed and not stressed halibut. Compared to several other species, Atlantic halibut has exceptional storing capacity both in sensory, chemical and microbiological terms. In this experiment, this is demonstrated by almost no development in pH, TVBN and viable bacteria values until after day 21. Farmed Atlantic halibut is likely to be accepted in the market as fresh fish at least one week longer than other white fish species (e.g. cod). CO_2 performed poorly as an anaesthetic and resulted in rapid onset of rigor mortis and high hardness values. Eugenol postponed onset of rigor mortis by 14 to 16 hours compared to CO_2 and gave a significantly lower measured hardness. Killing by a blow to the head resulted in increased variation in onset time and development of rigor mortis. Small differences were found in haem iron muscle residues among the groups, but halibut killed by a blow to the head were significantly better bled than fish anaesthetized with CO_2.
Descriptors: fish culture, seafood, quality control, slaughter, fish storage, storage life, *Hippoglossus hippoglossus*, Atlantic halibut
ASFA; Copyright © 2003, FAO

Annonymous. (1997) **Welfare of fish at slaughter**. *Trout News*. 24:42-44
The report of a workshop sponsored by the Humane Slaughter Association and the British Trout Association. The industry view, the veterinary view, research on slaughter methods, and industry requirements were covered.
Descriptors: fisheries, slaughter, animal welfare
ASFA; Copyright © 2003, FAO

Bernoth EM, Wormuth HJ (1991) **Tierschutzaspekte bei der Toetung von Fischen. [Aspects of animal welfare in killing fishes.]** *Bundesgesundheitsblatt*. 34(1):8-10 (In German)
ISSN: 0007-5914
Descriptors: fishes, destruction of animal, slaughtering, animal welfare, animal health, animals, aquatic animals, aquatic organisms, harvesting, methods, processing

Bernoth EV (1990) **Schaedigung von Fischen durch Turbinenanlagen. [Fish damage by turbines.]** *Deutsche Tieraerztliche Wochenschrift*. 97(3):161-164 (In German with English summary)
NAL Call No. 41.8 D482
Descriptors: fishes, lesions, water power, machinery, animal welfare, animal health, animals, aquatic animals, aquatic organisms, energy sources, equipment, injurious factors, natural resources

Bernoth EM von, Wormuth HJ (1990) **Tierschutzaspekte bei der Toetung von Fischen. [Animal protection aspects on killing of fish.]** *Deutsche Tieraerztliche Wochenschrift.* 97(4):154-157 (In German with English summary)
NAL Call No. 41.8 D482
According to the Animal Protection Law (1986) fish are to be killed by methods which do not cause pain. However, the regulations do not cover the killing of non-food fish. A questionnaire, conducted among 85 fish scientists, revealed that single fish should be killed by a blow on the head, and larger numbers by electrical methods or by use of chemicals. Decapitation was proposed for eels. A regulation from 1936 stipulates the methods for the slaughtering of food fish. Mechanical or electrical stunning is compulsory except for eel and flatfish. The questionnaire showed that in general the present legal regulations are sufficient for the slaughtering of fish with the exception of eels. The commercially available apparatus for stunning and killing do not always fulfill the requirements of animal protection, slaughtering technology and safety for the user. Official testing of these apparatus as well as the evaluation of new methods - like CO_2 -stunning - are necessary in order to prevent the use of methods which are feasible, but do not fulfill animal welfare, especially for eel.
Descriptors: fishes, slaughtering, destruction of animals, animal welfare, animal health, animals, aquatic animals, aquatic organisms, harvesting, methods, processing, government policy, mortality causes, commercial species, fishery technology, fish handling, processing fishery products, slaughter, fish, animal welfare
ASFA; Copyright © 2003, FAO

Bretzinger C (2001) **Einfluss unterschiedlicher Betaeubungsmethoden auf Stressbelastung und Produktqualitaet bei der Regenbogenforelle (*Oncorhynchus mykiss*) [Influence of different pre-slaughter stunning methods on stress reaction and product quality of rainbow trout (*Oncorhynchus mykiss*)].** *Muenchen FRG Hieronymus.* 189 pp. *(In German)* Dissertation. (Dr. vet. med.); Incl. 20 pages refs.
In the present study the stunning of rainbow trout (*Oncorhynchus mykiss*) via pulsed direct current electroshock, carbon dioxide exposition without and with oxygen enrichment and anaesthesia with the fish anaesthetic AQUI-S in comparison to stunning with a blow on the head was examined with respect to animal welfare and product quality of fish as food. The aspect of animal welfare was assessed by determination of the parameters respiration, aversive reactions, mucus secretion and the time period until fish enter anaesthesia as well as the blood parameters adrenaline, noradrenaline and packed cell volume. Product quality was evaluated by registration of carcass lesions, hemorrhages and fillet colour. All five methods proved to be effective for anaesthesia of trout. The blow on the head is the preferable method to stun consumption-sized rainbow trout if only a small number of fish is to be slaughtered. The electroshock method was not suitable for stunning larger numbers of trout for a sufficient period of time. Introduction of pure CO_2 gas, respectively CO_2 and CO_2 into water, also had to be considered as not suited because the fish showed strong signs of dyspnoe and aversive reactions until entering anaesthesia for on average 10 min., respectively 12 min. Oxygen enrichment obviously did not reduce the stress influence on trout. Applying the fish anaesthetic AQUI-S in a dose rate of 25 ppm enabled to stun rainbow trout for the slaughtering process. During an average narcosis period of 9 min. no signs of dyspnoe or an aversive reaction were recorded. Therefore, AQUI-S can be recommended -- without considering food and drug legislation -- as an alternative method for stunning large amounts of fish, which fulfills the requirements of animal welfare. The blood parameters adrenaline and noradrenaline showed significant differences between the stunning methods. The blow to

the head and anaesthesia with AQUI-S gave -- comparable with each other -- the lowest values for adrenaline and noradrenaline. At the end of electronarcosis intermediate amounts, after carbon dioxide exposition and CO_2/CO_2 narcosis the highest catecholamine concentrations were measured. The latter indicates a strong stress reaction. The packed cell volume values were lowest after percussive stunning. For the remaining methods higher values were determined. Visible alterations regarding the product quality of the carcasses were registered in trout being stunned by electroshock, which showed "current marks" and hemorrhages. The results of the colour measurements did not give hints on negative effects on product quality in dependence of the stunning method.
Descriptors: slaughter, anaesthetics, quality assurance, human food *Oncorhynchus mykiss*

Coutant, C.C., Whitney, R.R (2000) **Fish behavior in relation to passage through hydropower turbines: A Review.** *Transactions of the American Fisheries Society.* 129(2):351-380

We evaluated the literature on fish behavior as it relates to passage of fish near or through hydropower turbines. Our goal was to foster compatibility of engineered systems with the normal behavior patterns of fish species and life stages such that passage into turbines and injury in passage are minimized. In particular, we focused on aspects of fish behavior that could be used for computational fluid dynamics (CFD) modeling of fish trajectories through turbine systems. Salmon smolts approaching dams are generally surface oriented and follow flow. They can be diverted from turbines by spills or bypasses, with varying degrees of effectiveness. Smolts typically become disoriented in dam forebays. Those smolts drawn into turbine intakes orient vertically to the ceilings but are horizontally distributed more evenly, except as they are affected by intake specific turbulence and vortices. Smolts often enter intakes while oriented with their heads upstream, but they may change orientation in the flow fields of the intake. Nonsalmonids most often enter intakes from the vicinities of shorelines, and they do so episodically, which suggests accidental capture of schools (often of juveniles or in cold water) and little behavioral control during turbine passage. Models of fish trajectories should not assume neutral buoyancy throughout the time period during which a fish passes through a turbine, largely because of pressure effects on swim bladders and the resulting compensatory behavior. Fish use their lateral line system to sense obstacles and to change their orientation, but this sensory response system may not be effective in the rapid passage times and complex pressure regimes of turbine systems. The effects of preexisting stress levels on fish performance in turbine passage (especially as they affect trajectories) are not known but may be important. There are practical limits of observation and measurement of fish and flows in the proximity of turbines that may inhibit the development of much information that is germane to developing a more fish friendly turbine. We provide recommendations for CFD modelers of fish passage and for additional research.
Descriptors: literature reviews, freshwater fish, anadromous species, migratory species, smolts, lateral line, orientation behaviour, biological stress, avoidance reactions, entrainment, hydroelectric power plants, rivers, fishways, fishery management, salmon fisheries, river fisheries, nature conservation, river engineering, reviews, animal welfare, human impact, hydropower turbines, fish passages, hydroelectric plants, fish behavior, literature review, salmon, turbines, fish migration, Salmonidae

Drawer K (1987) **Die Praxis der Schlachttierbetaeubung aus der Sicht des Tierschutzes. [Animal welfare in stunning of slaughter-animals.]** *Tieraerztliche Umschau.* 42(11):878-885. (In German)
NAL Call No. 41.8 T445
Descriptors: domestic animals, stunning, animal welfare, animal health, animals, fishing methods, fishing operations, harvesting, methods, processing, slaughtering, fish welfare

Drawer K (1987) **Das Schlachten von Tieren im geaenderten Tierschutzgesetz [Slaughtering of animals in the new animals welfare act].** *Deutsche Tieraerztliche Wochenschrift.* 94(2):106-107. (*In German*)
NAL Call No. 41.8 D482
Descriptors: domestic animals, slaughtering, animal welfare, laws, stunning, animal health, animals, fishing methods, fishing operations, harvesting, legislation, methods, processing, slaughtering

Drosse H (1994) **Die "Lebendhaelterung" gefangener Fische im Setzkescher. [Fish farming landing nets.]** *Rundschau fuer Fleischhygiene und Lebensmittelueberwachung.* 46(9):207-209
ISSN: 0178-2010
Descriptors: game fishes, angling, animal welfare, fish cages, legislation, fishing rights, aquaculture equipment, equipment, fishing methods, legal rights

Grandin T (1985) **Cardiac arrest stunning of livestock and poultry**. Eds: Fox MW, Mickley LD. *Advances in animal welfare science.* Martinus Nijhoff, Dordrecht (Netherlands), 1986, p. 1-30
NAL Call No. HV4701 A34
Descriptors: livestock, poultry, stunning, heart, anatomy, animal anatomy, animals, birds, body parts, cardiovascular system, domestic animals, domesticated birds, fishing methods, fishing operations, harvesting, methods, processing, slaughtering, vertebrates

Gregory NG (1998) ***Animal Welfare and Meat Science.*** CAB International.Wallingford, OX10 8DE, UK. 298 pp.
NAL Call No. HV4731 G74 1998
It is recognised that careful and humane treatment of slaughter animals at the abattoir influences the quality of their meat, apart from humane considerations, and supporting evidence is assembled here. Correct slaughter procedure and appropriate abattoir installations are dealt with. There are separate chapters on cattle, sheep, pigs, poultry and fish, to deal with special requirements for each species.
Descriptors: slaughter, livestock, fish, animal welfare, meat quality
Copyright © 2003, CAB International.

Gregory NG (1996) **Welfare and hygiene during preslaughter handling.** *Meat science* 43 (suppl.):S35-S46 Massey University, Palmerston North, NZ.
NAL Call No. TX373.M4
Descriptors: meat quality, slaughter, animal welfare, stress, abattoirs, carcass quality, damage, food hygiene, literature reviews, glycogen, muscles, metabolism, handling, fish, pigs, beef cattle

Kestin SC, Vis JW van de, Robb DHF (2002) **Protocol for assessing brain function in fish and the effectiveness of methods used to stun and kill them.** *The Veterinary Record : Journal of the British Veterinary Association.* 150(10):302-307
NAL Call No. 41.8 V641
Descriptors: marine fishes, freshwater fishes, stunning, slaughter, animal welfare, brain, physiological functions, neurophysiology, evaluation, anesthesia, animal behavior

Kestin S (1993) **Welfare of fish at harvest**. *Trout News*. 17:28-30
Descriptors: fishes, fish culture, death, animal welfare, slaughter, *Oncorhynchus mykiss*, aquaculture, bony fishes, developmental stages, fish, salmonoidei

Knierim U (1996) **Die Tierscutz-Schlachtverordnung. [The animal welfare regulations at slaughter.]** *Deutsche Tierarztliche Wochenschrift* 103(2):52-54 (In German with English summary)
NAL Call No. 41.8 D482
The animal welfare regulation on the slaughter of animals, existing only as a draft for the time being, is designed not only to transpose EC-legislation into national law but also to update and strengthen preconstitutional national legislation on this matter. For a wide area related to the slaughter or killing of animals, animal welfare requirements are put in concrete terms. Among the topics belonging to this area are the theoretical and practical knowledge of the personnel, the handling of animals before slaughter or killing, stunning, the control of its efficacy and the permessibility of certain stunning or killing methods. Not only livestock but also, for example, fur animals and fish are concerned. In practice it will take some efforts in order to attain compliance with the provisions of the animal welfare slaughter regulation.
Descriptors: regulations, stunning, poultry, legislation, slaughter, animal welfare, cattle, pigs, sheep, fishes

Lambooij E, Vis JW, van de Kloosterboer RJ, Pieterse C (2002) **Welfare aspects of live chilling and freezing of farmed eel (*Anguilla anguilla*): neurological and behavioural assessment.** *Aquaculture*. 210 (1/4):159-169
NAL Call No. SH1A6
The overall objective of the study was to evaluate a slaughter method of eels, which consisted of chilling until their body temperature was <5 °C for stunning, and subsequently placing them in cold brine at -18°C for 15 min for killing. Three distinct experiments and a control were performed. First, 19 eels with an average live weight of 758 ± 44 g were restrained and equipped with EEG, ECG electrodes and a temperature sensor inside the body. Then, they were placed in the ice water. Indices for the induction of unconsciousness and insensibility were the appearance of theta and delta waves and no response on pain stimuli, which disappeared at a body temperature of 8.0 ± 2.1°C after 12 ± 5 min in 15 eels. The responses to pain stimuli did not disappear in three eels. Within a confidence level of 95%, the percentage of eels that was not effectively stunned during the procedure in ice water of <5°C was at least 5%. The heart rate decreased from 24 ± 10 beats/min (n=14) to 7 ± 4 (n=11) and became irregular during cooling down. When placed in the brine water of -18°C, the EEG showed rapid and extreme depolarization of the membranes, which started after 27 ± 17 seconds (n=18). The ECG showed fluttering of the heart in all eels. None of the eels recovered after this procedure. For 10 eels with an average live weight of 128 ± 27 g, it was observed that the body temperature decreased from 17.1 ± 0.6 to 4.0 ± 0.5°C in the ice water. After 15 min in the brine water of -16.1 ± 2.2°C, the body temperature decreased to -3.1 ± 2.3°C. Finally, three groups of 7 eels and 8 single eels were placed in ice water of -0.0 ±

0.1°C. The observation of unrestrained eels revealed four phases. Animals were (1) swimming around in the water, (2) attempting to escape from the ice water, (3) pressing their nose to the wall or corner while showing clonic muscle cramps, and finally (4) breathing only, while all other muscle activity was totally suppressed. Afterwards, they were transferred to cold brine at -18°C, and none of the eels recovered. The eight control eels, which were transferred to water at 18°C, swam around, except for one that was lying in an S-shape position at the bottom. After 570 and 605 seconds, two eels tried to escape from the box. The obtained results showed that the eels, which were transferred from water at 18°C to ice water, might be stressed, a specific behaviour and an irregular heart rate were observed. From an animal welfare point of view, it is therefore not recommended to stun eels by live chilling. Moreover, at least 5% of the eels will not be stunned at a body temperature of <5°C. Placing eels in brine water of -18°C is an effective method to kill the eels before slaughter. However, it cannot be recommended to place conscious eels in cold brine water, because it takes more than 27 seconds before unconsciousness may be induced.
Descriptors: animal behaviour, animal welfare, body temperature, chilling, freezing, heart rate, neurology, pain, slaughter, stunning, eels, *Anguilla*, Anguillidae, Anguilliformes, Osteichthyes, fishes, diadromous fishes, aquatic animals, aquatic organisms

Marx H, Brunner B, Weinzierl W, Hoffmann R, Stolle A (1997) **Methoden zur Betaeubung von Suesswasserfischen : Einfluss auf die Fleischqualitaet und Tierschutzaspekte. [Methods of stunning freshwater fish: impact on meat quality and aspects of animal welfare.]** *Zeitschrift fuer Lebensmittel Untersuchung und Forschung.* 204(4): 282-286 (In German with English summary)
NAL Call No. TX341 Z45
Taking into account aspects of meat quality and animal welfare, three methods of stunning fish were compared: a manual technique (blow on the head, stab in the neck), one using electricity and one using CO_2. The following results were obtained using eel, carp and trout. From the viewpoint of animal welfare, the effects on the fish were judged.
Exitation and mucus secretion as well as the time taken for the fish to be anaesthetized were recorded. With manual and electricalstunning, all fish were anaesthetized almost immediately, while using CO_2 it took 3.2 min (trout), 9.2 min (carp) and 109.7 min (eel) on average. After slaughter and after 3 and 8 days of storage on ice, the fish meat quality parameters, i.e. pH value, water-holding capacity and rigor mortis, were measured. CO_2 stunning gave rise to the lowest pH values and water-holding capacities. Rigor mortis in carp and eel advanced the most. Testing of raw and prepared fish was performed by a panel assessing organoleptic properties. In many cases, fish anaesthetized manually were ranked to be better than those in the other groups.
Descriptors: freshwater fishes, fish, stunning, time, quality, pH, water holding capacity, postmortem changes, organoleptic properties, animal welfare, eels, carp, trout, animal products, chemicophysical properties, diadromous fishes, fishery products, fishes, freshwater fishes, quality

Marx H, Brunner B, Weinzierl W, Hoffmann R, Stolle A. (1996) **Comparative investigations on different methods for stunning fish with special regard to meat quality parameters.** *Proceedings of the conference of IIR Commission C2, Bordeaux Colloquium Refrigeration and Aquaculture Froid et Aquaculture Colloque de Bordeaux, compte rendu de la reunion de la Commission C2 de l' IIF Paris France Institut International du Froid.* pp. 199-206

NAL Call No. TP490 S34

Taking into account aspects of meat quality and animal welfare, three methods for stunning fish were compared: manually (blow on the head, stab in the neck) with electricity and using CO_2. The following results were obtained for eel (n = 72), carp (n = 120) and trout (n = 54). From the view of animal welfare, the effects on the fish were judged. Excitation and mucus secretion, as weIl as the period of lime until the fish were in anaesthesia were recorded. With manual and electrical stunning, aIl fish were anaesthetized almost immediately, while using CO_2, it takes 3.2 min (trout), 9.2 min (carp) and 109.7 min (eel), on average. After slaughter, after three and eight days of storing the fish on ice, the meat quality parameters, pH value, water holding capacity and rigor mortis were measured. CO_2 stunning showed the lowest pH-values and water holding capacities; also, rigor mortis in carp and eel advanced most. Testing of raw and prepared fish was performed by a sensoric team. In many cases, fish anaesthetized manually were ranked better than the other groups. The findings indicate that CO_2 was not appropriate for stunning carp and eel. Electrical stunning, with some improvements, could meet the requirements of meat quality and animal welfare.

Descriptors: processing fishery products, anaesthesia, slaughter, quality, *Oncorhynchus mykiss, Cyprinus carpio, Anguilla anguilla*

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Muenkner W, Kuhlmann H, Oehlenschlaeger J (2000) **Sensibilitaet von Seefischen an Bord. Teil 2: Demersale und pelagische Fischarten aus Schleppnetzfaengen in der Nord und Ostsee. [Investigations on the sensitiveness of sea water fish on board -- Part 2: Demersal and pelagic fish species of the North and Baltic Sea.]** *Inf Fischwirtsch Fischereiforsch.* 47(2):97-101 (In German)

The sensitiveness of different demersal and pelagic fish species of 70 hauls in the North and Baltic Sea in water depths of 60 to 250 m and 15 to 80 m, respectively, amount of catch of 100 to 3500 kg and trawling times of 0,5 to 6 h on board of the FRV Walther Herwig III was investigated. Some demersal fish species, e.g. saithe (*Pollachius virens*), were even still sensitive, when caught at a water depth of 250 m at a trawling time of 1,5 h. Generally the number of sensitive fishes was reduced with increasing water depth, amount of catch, trawling time and following storage of the catch on board. Among demersal fishes the species without swimbladder and flat fishes were clearly more resistent to mechanical stress. On the contrary, pelagic fish species were generally less robust. After trawling times of 2 h no sensitive animals were observed. In some fisheries there are mixed catches of demersal and pelagic fish species with different sensitiveness. In commercial fisheries, there is therefore under animal welfare aspects for the time being, no prospect for an improvement of the catching and slaughtering procedure on board.

Descriptors: biological stress, bottom trawls, midwater trawls, total mortality, *Pollachius virens, Gadus morhua, Clupea harengus, Melanogrammus aeglefinus,* ANE

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Neukirch M (1994) **Legal and animal welfare aspects of the killing of fish. [Uber rechtliche und tierschutzrelevante Aspekte bei der Totung von Fischen.]** *Deutsche tierarztliche Wochenschrift* (Germany). 101(8):316-319

NAL Call No. 41.8 D482

The selection of methods for killing fish is determined by the number of fish to be killed, their utilization and the existing laws. No regulations exist for killing non-food fish. The methods for killing food-fish, however, are stipulated in a regulation from 1936. Mechanical or electrical stunning is obligatory except for flatfish and eel. Single fish should be stunned

by a blow on the head, followed immediately by slaughtering or bleeding to be sure that the fish is really dead. When larger numbers of fish should be killed for food production only electrical methods are allowed as alternative. Chemicals can be used for killing non-food fish, the non-pollutant destruction of dead fish and chemical-contaminated water, however, has to be guaranteed. The methods are discussed with respect to practicability and animal welfare.
Descriptors: animal welfare, fisheries, legislation and jurisprudence, fishes, physiology, electric stimulation, Germany

Nowak D (1989) **Tierschutzrelevante Aspekte bei Halterung, Verkauf und Totung von Susswasserspeisefischen. [Welfare aspects of the holding, selling and killing of freshwater fishes for food.]** *Rundschau fur Fleischhygiene und Lebensmitteluberwachung* 41(7):139-140 (In German)
Descriptors: animal welfare, aquaculture, fish farming

Oehlenslager J, Kestin SC, Tejada M, Sorensen NK, Torrissen OJ, Nesvabda P, Van Rijsingen LCM (1998) **Optimisation of harvest procedures of farmed fish with respect to qualify and welfare.** *Third european marine science and technology conference MAST conference, Lisbon, 23 27 May 1998: Project synopses Vol 6: Fisheries and Aquaculture FAIR: 1994 98, selected projects from the research programme for Agriculture and Fisheries including agro industry, food technology, forestry, aquaculture and rural development FAIR. Luxembourg Luxembourg European Commission DG 12 Science, Research and Development.* 6:244-245
In the last decade consumers have become much more aware of product quality. This also includes the production and processing of animals under humane conditions. The greater awareness has led to an increased focus by processors on the quality of farmed fish. For some fish species, harvest conditions, especially slaughter, have been reported to have an adverse affect on flesh quality. This may result from the effects of stress, for example when the killing method is not instantaneous, in which case the welfare of the fish will also be affected. Preliminary results indicate that methods which kill fish rapidly can result in an improvement in quality and a reduction of stress, thereby also leading to an improvement in welfare. The objectives of the proposed study are therefore twofold: (1) the optimisation of the slaughter process of farmed fish with respect to quality and welfare, and (2) the automation of these optimum processes. The study will be undertaken on salmon (*Salmo salar*), gilt head bream (*Sparus aurata*) and eels (*Anguilla anguilla*). These three model species have been selected as they differ in their physiology. They will enable comparisons of species that require well oxygenated water (salmon) vs. less oxygenated water (eel) and that live in salt water (gilt head bream) vs. fresh water (eel). The differences in physiology are likely to be important for instantaneous killing methods and, when instantaneous killing is not possible, stunning prior to killing. Analytical and biochemical measurements will be used to assess eating and processing quality immediately post mortem and during subsequent handling and storage. The welfare aspects of killing methods will be evaluated by measuring brain activity in combination with observations of behaviour (using video recordings of fish in tanks) to determine how quickly death occurs of a state of insensibility is reached. Based on the results obtained, the automation of the optimum procedures, including use of folgrade anaesthetics, will be investigated with respect to compatibility with further processing, textural and sensory analysis. For the automation of slaughter processes which would be feasible fore use by SMEs, two SMEs and an association of fish farmers are involved in the project.
Descriptors: harvesting, fishery products, quality control, fish ponds
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Ottera H, Roth B, Torrissen OJ (1999) **Do Killing Methods Affect the Quality of Atlantic Salmon?** *Farmed Fish Quality.* (Eds.) Kestin SC, Warriss PD. International Conference on Farmed Fish Quality, [n.p.], 7-9 Apr 1999. Blackwell Science Ltd. p.398-399.
NAL Call No. SH151 F37 2001
The methods used for stunning and killing fish species in aquaculture have recently received a lot of attention, from an ethical point of view - does the fish suffer unnecessary pain during the process - and also from a product quality point of view. These two aspects were the rationale for the EU-project "Optimization of harvest procedures of farmed fish with respect to quality and welfare - FAIR CT97-3127 FAQUWEL." Here we present some of the preliminary results on product quality of Atlantic salmon (*Salmo salar*) as affected by killing method. We are evaluating four methods for killing the salmon: Sedation by COZ, followed by gill-cutting, Electro-stunning, followed by gill-cutting, Brain destruction by a pin-bolt machine, followed by gill-cutting, and Direct gill-cutting. Sedation by CO_2 or direct gill-cutting are the most commonly used methods in the aquaculture industry, but recently the interests in alternative methods have evolved, and commercial use of electro-stunning and various types of brain destruction techniques are in development. We also did a simple trial on using laughing gas N_2O as a sedative, but that apparently had no effect on the salmon. Major evaluation criteria included development of rigor mortis and pH during storage on ice, cortisol measurements as an indicator of stress during slaughter, and various product quality measurements taken on raw fish stored four days on ice. As expected, fish killed by the methods supposed to be most 'brutal', use of CO_2 or direct gill-cutting, also went into rigor mortis first, and had the highest rigor index. Similarly, they seemed to have the most rapid initial drop in pH. Both these factors indicate that the use of these traditional killing methods for salmon may be inferior to new methods like electro-stunning and pin-bolting. Differences in ultimate flesh quality, measured on raw fish stored four days on ice are, however, more difficult to find. Preliminary data analysis does not indicate differences between killing methods on fillet colour; on the other hand there are indications that fish bled to death after gill-cutting had softer fillets (measured as Warner-Bratzler shear force). Further analysis and experiments will go into more detail in evaluating salmon quality as a function of killing method.
Descriptors: fish culture, seafood, quality control, slaughter, heading, *Salmo salar*

Robb DHF, Kestin SC (2002) **Methods used to kill fish: field observations and literature reviewed.** *Animal Welfare.* 11(3):269-282
Wheathampstead, U.K. : Universities Federation for Animal Welfare.
NAL Call No. HV4701.A557
Descriptors: fishes, fish farming, slaughter, asphyxia, stunning, carbon dioxide, evisceration, animal welfare, food quality

Robb DF, Wotton SB, Van deVis JW (2002) **Preslaughter electrical stunning of eels.** *Aquaculture Research.* 33(1):37-42
NAL Call No. SH1.F8
The current procedures for slaughtering European eels (*Anguilla anguilla*) for food are very slow and cause suffering. Although there is little legislation for protecting the welfare of fish at slaughter, the legislation covering farmed mammals and birds at slaughter is well defied, requiring that these animals be rendered insensible immediately or without fear or pain prior to being killed. For many mammals and birds this can be achieved using an electrical stun, which is then followed by a procedure that actually kills them, such as exsanguination. This

paper reports the investigation of the possibility of using electricity to stun eels, rendering them insensible to pain. Using 1 s duration alternating currents at 50 Hz applied directly across the head of the fish, it was shown that it was possible to stun the fish with currents of 0.1 A and above. Increasing the applied current increased the length of the period of the stun. When the duration of the application of the current was increased to 30 s it was found that the fish could be killed using currents between 0.50 A and 0.95 A. These results show that it is possible to use electricity to instantly stun eels and also to kill them by using longer duration currents. The use of preslaughter electrical stunning at slaughter could allow the welfare of these fish at slaughter to be improved greatly.
Descriptors: processing fishery products, aquaculture products, electricity, harvesting, *Anguilla anguilla*, eels, slaughter, welfare
ASFA; Copyright © 2003, FAO

Robb D (1997) **Welfare of fish at slaughter**. *Fish Farmer*. 20(2):7-8
NAL Call No. SH151 F57
Descriptors: aquaculture, fish culture, stunning, slaughtering, equipment, anaesthesia, carbon dioxide, Atlantic salmon, *Oncorhynchus mykiss*, fish, quality, animal welfare, animal products, aquaculture, bony fishes, diadromous fishes, fishery products, oxides, salmon, salmonoidei

Schulz D (1978) **Zum tierschutzgerechten Betauben und Toten von Fischen. [Humane stunning and killing of fish.]** *Du und das Tier*. 8(1):31-33 (In German)
ISSN: 0341-5759
Descriptors: ammonia, electricity, anaesthetics, slaughter, animal welfare, fishes

Southgate P, Wall T (2001) **Welfare of farmed fish at slaughter.** *In practice*. 23 (5):277-280, 282-284 London : British Veterinary Association.
NAL Call No. SF601.I4
Descriptors: fishes, fish farming, animal welfare, slaughter, fish, quality

Tejada M, Huidobro A, Pastor A (2001) **Slaughter Methods Affecting Adenosine Triphosphate and Derivatives in Chilled Stored Gilthead Seabream (*Sparus auratus*).** (Eds:) Kestin SC, Warriss PD. *Farmed Fish Quality*. Osney Mead Oxford OX2 0EL UK.
NAL Call No. SH151 F37 2001
Animal welfare is becoming an increasingly important part of consumer perception of quality; however, different fish slaughter procedures can affect the final quality of the fish. Given the diversity of slaughtering methods used in farmed fish, it is essential to assess how these methods affect fish quality during chilled storage. Breakdown of adenosine-5'-triphosphate (ATP) and derivatives and the ratio between them (as K value) are early indicators of changes in post-mortem fish; they are widely used to set safe consumption limits for raw fish and as indices of freshness of chilled fish or raw material for gels. Our aim was to determine whether gutting immediately after death and different methods of slaughter alter the evolution of these compounds or their ratio during chilled storage in gilthead seabream (*Sparus auratus*) killed by immersion in ice-water slurry, asphyxia in air, anaesthesia (AQUI-S™) followed by a blow on the head, and just a blow on the head. The fish was stored with ice flakes whole (w) or gutted (g) for a maximum time of 29 days. Forty kg (approximately 130 fish/lot) was used for each slaughter method and post-mortem treatment. In all lots ATP rapidly degraded to inosine monophosphate (IMP) during chilling storage, leaving no appreciable amounts of adenosine-5'-diphosphate (ADP) or adenosine-5'-

monophosphate (AMP). Dephosphorylation of IMP was slow and progressive. Inosine (Ino) and hypoxanthine (Hx) increased gradually over storage in all lots with no significant differences ($p < 0.05$), but Ino tended to accumulate and Hx tended to stabilize by the end of storage. The molar ratio Hx : Ino was < 5:1 throughout the period, and therefore this species was classified as intermediate. None of the lots attained K values > 20% before seven days in chilled storage, which means that sashimi grade (raw fish) for this species was longer than for other commercial species. Maximum K values were established at 50-60% at the end of the storage period, well past the sensory limit, and around 35% (w) and 25% (g) of lots when the sensory evaluation was still within the limits.
Descriptors: fish culture, seafood, quality control, chilled products, slaughter, ATP, fish storage, *Sparus aurata*

van de Vis JW, Oehlenschlaeger J, Kuhlmann H, Muenkner W, Robb DHF, Schelvis Smit AAM (2001) **Effect of the commercial and experimental slaughter of eels (*Anguilla anguilla* L.) on quality and welfare**. (Eds:) Kestin SC, Warriss PD. *Farmed Fish Quality*. Osney Mead Oxford OX2 0EL UK. Blackwell Science Ltd. pp. 234-248
NAL Call No. SH151 F37 2001
In fish the welfare ante-mortem and the quality of the flesh post-mortem can be adversely affected by farming and harvest conditions. Farming conditions comprise, amongst others, water quality and stocking density. Harvest consists of crowding, catching, transport, lairage and slaughter, and is one of the most intense stressors in fish farming (Thomas et al. 1999). There are similarities in anatomy, (neuro) physiology and behaviour between fish, mammals and birds (Kestin 1994; FAWC 1996; Wendelaar Bonga 1997; Wiepkema 1997). Therefore, it is likely that the welfare of fish can potentially be similarly adversely affected by husbandry and harvesting conditions. In red and white meat animals handling and slaughter procedures may have profound effects on the course of chemical changes post-mortem and consequently on the quality of the fresh and processed meat. There is some evidence that in fish a similar relationship exists between harvest procedures and aspects of quality such as water holding capacity, texture and keeping quality (Azam et al. 1989; Iwamoto et al. 1990; Proctor et al. 1992a,b; Lowe et al. 1993; Kals et al. 1995; Templeton 1996; Marx et al. 1997; Sigholt et al. 1997; Thomas et al. 1999). However, not all researchers have observed effects on sensory parameters attributable to harvest methods used (see Chapter 20 by Dave Robb). Nevertheless, it is known that both handling and slaughter methods used may affect post-mortem biochemical.
Descriptors: fish culture, seafood, quality control, processing fishery products, heading, slaughter, *Anguilla anguilla*

Wall AJ (2001) **Ethical Considerations in the Handling and Slaughter of Farmed Fish**. (Eds:) Kestin SC, Warriss PD. *Farmed Fish Quality*. Osney Mead Oxford OX2 0EL UK. Blackwell Science Ltd. pp. 108-115
NAL Call No. SH151 F37 2001
This chapter will discuss preslaughter management practices and slaughter methods, and how these might influence both the welfare of the fish and the quality of the final product. Generally speaking there is no conflict between the welfare of the fish and quality. Good welfare and good quality go hand in hand.
Descriptors: fish culture, seafood, quality control, fish handling, slaughter, processing, fishery products

Wormuth HJ (1987) **Das betaeubungslose Schlachten (Schaechten) aus neuer tierschutzrechtlicher Sicht. [Slaughtering according to Jewish rites in the view of the new animal welfare act.]** *Deutsche Tieraerztliche Wochenschrift.* 94(2):107-109. (In German)
NAL Call No. 41.8 D482
Descriptors: domestic animals, slaughtering, animal welfare, laws, stunning, kosher slaughter, animal health, animals, central europe, europe, fishing methods, fishing operations, harvesting, legislation, methods, processing, slaughtering

Wormuth HJ (1986) **Das betaeubungslose Schlachten (Schaechten) aus neuer tierschutzrechtlicher Sicht. [Slaughtering without stunning (kosher butchering) from the point of view of the law of animal welfare.]** *Rundschau fuer Fleischuntersuchung und Lebensmitteluebenwachung.* 38(11):224-225. (In German).
ISSN: 0178-2010
Descriptors: domestic animals, slaughtering, animal welfare, laws, stunning, animal health, animals, fishing methods, fishing operations, harvesting, legislation, methods, processing, slaughtering

Web Resources:

Aspects of Animal Welfare and Aquaculture -A Compendium of Selected Literature by Richard D. Moccia and Kristopher P. Chandroo; Aquaculture Centre, University of Guelph, Guelph, Ontario, Canada
http://www.aps.uoguelph.ca/~aquacentre/aec/publications/welfare-bib.html

Center for Critical Quality Attribute Determination in Muscle Foods
Research program from July 1st 1998 to October 2001
Supported by SJVF, Denmark
http://www.dfu.min.dk/qmf/Information.htm

Harvest Hyperactivity and Post-Mortem Change In Tuna
Southern Bluefin Tuna Aquaculture Subprogram Newsletter, June 2000
http://www.sardi.sa.gov.au/pages/aquatics/aqua/other/sbt_june_2000.html

Processing and Marketing Aquacultured Fish
Joel M. Regenstein
Northwest Regional Aquaculture Center, NRAC Factsheet No. 140, 1992
http://aquanic.org/publicat/usda_rac/efs/nrac/nrac140.pdf

Rested Harvesting using AQUI-S
DJ Bell, AR Jerrett, J Holland
AQUI-S New Zeland, LTD.
http://www.aqui-s.com/version_common/common_rested.html

3.2.6.3. Holding & Transport

Bohl M (1978) **Zur tierschutzgerechten Halterung von Fischen.[Humane holding of fish].** *Du und das Tier.* 8(1):26-28 (In German)
ISSN: 0341-5759
Descriptors: stress, animal feeding, conditioning, water, fish farming, animal welfare

Bowszys J, Kupren B (1986) **Badania wstepne nad przystosowaniem podnosnika hydropneumatycznego do transportu ryb. [Preliminary studies on adoption of a hydropneumatic elevator for fish transport.]** *Acta Academiae, Agriculturae ac Technicae Olstenensis, Aedificatio et Mechanica.* 15:65-78 (In Polish with English and Russian summary)
No mechanical injuries to the fish resulted. Oxygen levels in the water remained within accepted tolerances.
Descriptors: aquaculture, elevators, animal welfare
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Collins C (1990) **Live-hauling warmwater fish**. *Aquaculture Magazine.* 16(4):70-76
NAL Call No. SH1 C65
Descriptors: animal welfare, water, oxygen, ammonia, antiinfective agents, antibiotics, anaesthetics, transport of animals, fishes, aquatic animals

Forsberg JA, Barton BA, Summerfelt RC (1999) **Effects of ram-air ventilation during transportation on water quality and physiology of walleye fingerlings.** *Stress in fish.* pp. 31-36
The Iowa Department of Natural Resources (IDNR) maintains walleye (*Stizostedion vitreum*) populations in most Iowa lakes and reservoirs by stocking fry or hatchery-raised fingerlings. Some fingerlings must be transported 6-7 hours from hatchery to stocking sites. IDNR personnel estimate that post-stocking survival of fingerling walleye transported for 6-7 h is substantially lower than survival of walleye tansported for 1h or less. The working hypothesis is that dissolved carbon dioxide (CO_2) accumulates in tank water as a result of fish respiration and a lack of tank ventilation. After being transported 6 h, fish seemed dazed, perhaps sedated from CO_2. An anesthetic effect may occur when CO_2 concentrations reach 100 mg/L. Also, supersaturated oxygen (O_2) concentrations were common, which can cause further increases in blood CO_2 levels by decreasing ventilation rate. If fingerlings are sedated upon stocking, they are at increased risk of predation. Ram-air ventilators (RAV) were installed in two hauling tanks and measured for their effect on water quality during a 6-h haul. Two unvented tanks on the same truck served as controls. In this study, tanks equipped with RAVs had superior water quality (lower CO_2, higher pH, and normoxic O_2) compared with control tanks without RAVs. Fish transported in RAV-equipped tanks had a significantly lower stress response (cortisol only) after loading than that of walleye in control tanks, but the differences were not significant after the haul. Measurement of blood pCO_2 and blood HCO_3 support the water quality findings that fish transported in the RAV tanks were exposed to less dissolved CO_2 than fish in the control tanks.
Descriptors: fish physiology, biological stress, ventilation, carbon dioxide, oxygen, water quality, environmental effects, *Stizostedion vitreum*
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Laboratory Animal Breeders Association of Great Britain Limited (LABA) and Laboratory Animal Science Association (LASA). (1993) **Guidelines for the care of laboratory animals in transit.** *Laboratory Animals* (England). 27(2):93-107
NAL Call No. QL55 A1L3
Descriptors: animals, laboratory, transportation, animal feed, animal welfare, cats, dogs, guinea pigs, hamsters, housing, animal, laboratory animal science, mice, primates, rats, fish

Ministry of Agriculture, Fisheries and Food, Scottish Office Agriculture, Environment and Fisheries Department, Welsh Office Agriculture Department (1997) ***Draft Guidance on the Welfare of Animals (Transport) Order 1997.*** Surrey, England: The Ministry. 69 p.
NAL Call No. KD3424.A33 1997
Descriptors: animals, animal welfare, fish, transport

Muzinic R (1970) **On the use of anaesthetics in the transportation of sardines.** *Studies and Reviews of the General Fisheries Council of the Mediterranean.* 47:1-23
The rate of mortality of non-selected sardines, exposed to 1:150 000 conc of tricaine methane sulfonate in open-system experiments increased rapidly and after 2 hr, by far exceeded 50 per cent. The mortality rate of the fish slowed down considerably when the sardines were transferred to a fresh anaesthetic solution at 30 min intervals using a conc of 1:150 000 tricaine methane sulfonate or compressed air when the temps were 20.8° and 21.7°C; transferring the sardines had the same effect on their mortality using chloral hydrate at both 1:1000 and 1:3 000 concs and the effect was even more notable at the latter concs. Similar procedure may be applied in transporting sardines, especially from distant localities for tagging and other experimental work. It is possible that some changes in the composition of the anaesthetic solution during the initial phase of transportation may be useful. With changes in the anaesthetic solution being made at 30-min intervals, lower concs of chloral hydrate were more advantageous. In standard anaesthesia experiments however, this was not so. In standard anaesthesia experiments with chloral hydrate, a rapid increase in mortality occurred at a decline of the O_2 conc to a point below 2 cc/1. In open system standard anaesthesia experiments using 1:150 000 tricaine methane sulfonate conc, the last sardine died at a temp ranging from 20.3° to 22.8°C and at a mean final O_2 value of 0.56 ± 0.46 cc/1. Chloral hydrate at 1:3 000 and 1:5 000 concs (and perhaps even lower ones) may replace tricaine methane sulfonate in transporting the sardines. The delicate state of the fish was shown by a marked variability of the mortality course within all the series of anaesthesia experiments and by a rather high mean final oxygen value and its great variation.
Descriptors: anaesthetics, tricane methane sulfonate, sardines, titration experiment, chloral hydrate, temperature, oxygen
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Nowak D (1989) **Tierschutzrelevante Aspekte bei Halterung, Verkauf und Totung von Susswasserspeisefischen. [Welfare aspects of the holding, selling and killing of freshwater fishes for food.]** *Rundschau fur Fleischhygiene und Lebensmitteluberwachung* 41(7):139-140 (In German)
Descriptors: animal welfare, aquaculture, fish farming

Schulz D (1974) **Tierschutz und Halterung sowie Transport von Fischen. [Animal welfare, containers and transport of fish.]** *Berliner und Munchener Tierarztliche Wochenschrift.* 87(18):359-362 (In German with English summary)
NAL Call No. 41.8 B45

Present methods of holding and transporting fish are discussed, the legal requirements mentioned, and methods suggested which satisfy the legal and humane conditions.
Descriptors: legislation, transport of animals, transport, animal welfare, fishes
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Stede M (1978) **Zur tierschutzgerechten Halterung von Fischen. [Humane holding of fish.]** *Du und das Tier.* 8(1):29 (In German)
ISSN: 0341-5759
Descriptors: stress, water, environmental temperature, fish farming, animal welfare

Varadi L, Hegyi A, Hopp B (2000) **Narcotics in the fish transportation [Narkotikumok alkalmazasa a halszallitasban].** *Halaszat.* 93(1):44-48
NAL Call No. 414.8 H12
Nowadays, by the spreading of the intensive way of fish breeding the individual and collective treatment of these animals has gained even more importance. It appears also in propagation, breeding technologies and health care as well as in transportation. Because of the basic anatomical and physiological makings of fish tasks can hardly be done when awake without threathening the health of life of these animals. This more intensive way of fish production in ponds has sharply increased the demand for more stocks of fish. In many cases the weakest point in fish breeding technologies is transportation.
Descriptors: fishes, transport of animals, animal welfare, anaesthetics, drugs, neurotropic drug, transport

Vollmann-Schipper F (1978) **Zum tierschutzgerechten Transport von Fischen. [Humane transport of live fish.]** *Du und das Tier.* 8(1):30-31 (In German)
ISSN: 0341-5759
Descriptors: stress, containers, water, transport of animals, animal welfare, fishes

Yin Bangzhong, Liu Qi, Liang Mengqing, Jiang Yaosen (1995) **On transportation of live sea fish.** *Shandong fisheries/Qilu Yuye. Yantai.* 12(2):25-26
ISSN: 1001-151X
The common method for transportation of live animal is aeration method, anaesthesia method and low temperature method. But using non-water lowering temperature to keep fish alive for transportation has many advantages such as big load amount, no contamination and high quality, etc. It is a developing tendency to use this method for transportation of live sea fish.
Descriptors: live storage, storage conditions, fish storage, cold storage, transportation
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Zhang Xiaolan, Lin Hong, Lou Weifeng, Xue Changhu (1993) **Studies on comprehensively raising the survival rate in the transport of the live fish.** *Shandong fisheries/Qilu Yuye. Yantai.* 10(6):12-14
ISSN: 1001-151X
In order to raise the survival rate in the transport of the live fish a positive crossing experiment ($L_9(3^4)$) was made to transport the live Jian-carp first immersed in water with the concentration of 100×10^{-6} carbonic acid and later taken out to be put into the closing container filled with water containing different concentrations of the selected NaCl, H_2O_2 and Terramycin. The result showed that in seven groups the survival rate of Jian-carp was 80% on an average, and only the survival rate of the sceond group reached 100% in the water

in proportion of 0.2% NaCl, 170ml H_2O_2 and 20 x 10^{-6} Terramycin. The positive relativation appeared apparently between the dissolved oxygen and the survival rate; although the oxygen resourse, the variety of anaesthetic and medicine could effected more and less on the survival rate, yet there was no marked difference between the factors and the concentrations effected on the survival rate.
Descriptors: transportation, fish handling, survival, live storage

Web Resources:

Aspects of Animal Welfare and Aquaculture - A Compendium of Selected Literature by Richard D. Moccia and Kristopher P. Chandroo; Aquaculture Centre, University of Guelph, Guelph, Ontario, Canada
http://www.aps.uoguelph.ca/~aquacentre/aec/publications/welfare-bib.html

Capturing, Handling, Transporting, Injecting and Holding Broodfish for Induced Spawning
RW Rottman, JV Shierman, FA Chapman
Southern Regional Aquaculture Center, *SRAC Publication No. 422*
http://agpublications.tamu.edu/pubs/efish/422fs.pdf

Marketing and Shipping Live Aquatic Products
http://www.nraes.org/publications/nraes107.html

Transportation of Warmwater Fish: Equipment and Guidelines
GL Jensen
Southern Regional Aquaculture Center, *SRAC Publication No. 390*
http://aquanic.org/publicat/usda_rac/efs/srac/390fs.pdf

Collecting Rainbowfishes
http://members.optushome.com.au/chelmon/Collect.htm

Transport of Fish and Crustaceans in Sealed Containers
SK Johnson
http://agpublications.tamu.edu/pubs/efish/1504a.pdf

3.2.6.4. Tagging

Adams NS, Rondorf DW, Evans SD, Kelly JE (1998) **Effects of surgically and gastrically implanted radio transmitters on growth and feeding behavior of juvenile chinook salmon.** *Transactions of the American Fisheries Society.* 127(1):128-136
NAL Call No. 414.9 AM3
We examined the effects of surgically and gastrically implanted radio transmitters (representing 2.3-5.5% of body weight) on the growth and feeding behavior of 192 juvenile chinook salmon *Oncorhynchus tshawytscha* (114-159 mm in fork length). Throughout the 54-d study, the 48 fish with transmitters in their stomachs (gastric fish) consistently grew more slowly than fish with surgically implanted transmitters (surgery fish), fish with surgery but no implanted transmitter (sham-surgery fish), or fish exposed only to handling (control fish). Growth rates of surgery fish were also slightly impaired at day 21, but by day 54 they were growing at rates comparable with those of control fish. Despite differences in growth, overall health was similar among all test fish. However, movement of the transmitter antenna caused abrasions at the corner of the mouth in all gastric fish, whereas only 22% of the surgery fish had inflammation around the antenna exit wound. Feeding activity was similar among groups, but gastric fish exhibited a coughing behavior and appeared to have difficulty retaining swallowed food. Because growth and feeding behavior were less affected by the presence of surgically implanted transmitters than by gastric implants, we recommend surgically implanting transmitters for biotelemetry studies of juvenile chinook salmon between 114 and 159 mm fork length.
Descriptors: tagging, feeding behaviour, mortality causes, telemetry, growth, feeding behavior, marking and tracking techniques, surgery, *Oncorhynchus tshawytscha*, growth, Oncorhynchus, Chinook salmon
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Benoit E, Laurent D, Mattei C, Legrand AM, Molgo J (2000) **Reversal of Pacific ciguatoxin-1B effects on myelinated axons by agents used in ciguatera treatment.** First meeting on Ichtyology in France, RIF 2000. Premieres Rencontres de l'Ichtyologie en France, RIF 2000. *Cybium. Paris.* 24(3): 33-40
ISSN: 1399-0974
Ciguatera fish poisoning is a distinctive form of ichthyosarcotoxism characterised mainly by gastrointestinal and neurological disturbances. The ciguatoxins, responsible for this poisoning, are complex polyethers produced by toxic strains of the dinoflagellate Gambierdiscus toxicus. These toxins are increased to dangerous levels for man during their transmission through herbivorous and carnivorous fish, various species being contaminated. The known molecular target of ciguatoxins is the voltagegated Na+ channel. During the action of these toxins, the permanent opening of channels, at the resting membrane potential, produces a continuons entry of Na+ ions in excitable tells causing a marked increase in membrane excitability and in cellular volume. To precise the neurocellular bases of the efficacy of some agents used in clinical and traditional treatments of ciguatera, their effects were studied on frog myelinated axons exposed to Pacific ciguatoxin-1B (CTX-1B). During the action of this toxin, the increase in axonal volume and membrane excitability was reversed by lidocaine (a local anaesthetic), by $CaCl_2$ and by hyperosmotic external solutions (containing D-mannitol, sucrose or tetramethylammonium chloride). The CTX-1B-induced hyperexcitability of the membrane was also reversed by extracts of Argusia argentea leaves

or Davallia solida rhizomes, used traditionally in New-Caledonia. It is concluded that the various agents studied are able to counteract the neurocellular effects of CTX-1B in myelinated axons. These results are of particular interest since they provide a scientific basis to understand the beneficial action of therapeutic agents used in the treatment of ciguatera fish poisoning.

Original Abstract: La ciguatera est une forme particuliere d'ichtyosarcotoxisme principalement caracterisee par des troubles gastro-intestinaux et neurologiques. Ce sont les ciguatoxines, polyethers complexes produits par des varietes toxiques du dinoflagelle Gambierdiscus toxicus, qui en sont responsables en se concentrant pour atteindre des doses dangereuses pour l'homme lors de leur transfert dans de nombreuses especes de poissons herbivores et carnivores. La cible moleculaire connue des ciguatoxines est le canal Na+ sensible au potentiel de membrane. Durant l'action de ces toxines, l'ouverture permanente des canaux au potentiel de repos de la membrane, produit une entree continue d'ions Na+ dans les cellules excitables ce qui augmente notablement l'excitabilite membranaire et le volume cellulaire. Dans le but de preciser les bases neurocellulaires de l'efficacite.de certains agents utilises dans le traitement clinique et traditionnel de la ciguatera, leurs effets ont ete etudies sur des axones myelinises de grenouille prealablement soumis a l'action de la ciguatoxine-1B du Pacifique (CTX-1B). L'augmentation du volume axonal et de l'excitabilite de la membrane, produite par cette toxine, a ete neutralisee par la lidocaine (anesthesique local), le $CaCl_2$, et les milieux extracellulaires hyperosmotiques contenant du D mannitol, du saccharose ou du chlorure de tetramethylammonium.L'hyperexcitabilite membranaire, produite par la CTX-1B, a egalement ete supprimee par les extraits de feuilles d'Argusia argentea ou de rhizomes de Davallia solida, utilises dans la medecine traditionnelle en Nouvelle Caledonie. En conclusion, les divers agents etudies sont capables de neutraliser les effets neurocellulaires de la CTX-1B au niveau des axones myelinises. Ces resultats sont particulierement interessants puisqu'ils apportent une base scientifique necessaire a la comprehension de l'action benefique des agents therapeutiques utilises de maniere encore empirique dans le traitement de l'ichtyosarcotoxisme de type ciguatera.

Descriptors: ciguatoxin, fish poisoning, ions, therapy

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Benzer TI, Raftery MA (1972) **Partial characterization of a tetrodotoxin-binding component from nerve membrane.** *Proceedings of the National Academy of Sciences, USA.* 69(12):3634-3637

NAL Call No. 500 N21P

Tecrodotoxin from Japanese puffer fish has been labeled with tritium and purified from the crude mixture obtained. The interaction between the purified [^{3}H]tetrodotoxin and membrane suspensions from the olfactory nerve of long-nosed garfish has been investigated by equilibrium dialysis. Tetrodotoxin binds to membrane suspensions with a dissociation constant K_D=8.3nM. The nerve preparation binds 42 pmol of [^{3}H]tetrodotoxin/g of wet tissue at saturating toxin concentrations. With various hydrolic enzymes, the binding component is shown to be a protein embedded in a phospholipid environment. The binding is inhibited below pH 4.0 and is not stable towards heat. Tetrodotoxin binding is not inhibited by the local anesthetic, procaine.

Descriptors: puffer fish, tecrodotoxin, nerve, membrane, fish, phospholipic environment

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Bidgood BF (1980) **Field surgical procedure for implantation of radio tags in fish.** *Fisheries Research Report, Fish and Wildlife Division.* (Alta., Canada), No. 20. 10pp.

Equipment and methods employed in surgically implanting radio tags in fish in the field are described. Anesthetic and recovery procedures that reduce or eliminate shock and stress are presented. Field and laboratory applications of the procedure are documented.
Descriptors: tagging, sonic tags, Pisces, Canada, Alberta, methodology, freshwater fish, electronic equipment, fatigue (biological), migrations
ASFA; Copyright © 2003, FAO

Blankenship HL, Leber KM (1995) **A responsible approach to marine stock enhancement.** *Uses and Effects of Cultured Fishes in Aquatic Ecosystems., American Fisheries Society, Bethesda, MD (USA),* 15:167-179
NAL Call No. SH3 A5
Declining marine fish populations worldwide have rekindled an interest in marine fish enhancement. Recent technological advances in fish tagging and marine fish culture provide a basis for successful hatchery-based marine enhancement. To ensure success and avoid repeating mistakes, we must take a responsible approach to developing, evaluating, and managing marine stock enhancement programs. A responsible-approach concept with several key components is described. Each component is considered essential to control and optimize enhancement. The components include the need to (1) prioritize and select target species for enhancement; (2) develop a species management plan that identifies harvest opportunity, stock rebuilding goals, and genetic objectives; (3) define quantitative measures of success; (4) use genetic resource management to avoid deleterious genetic effects; (5) use disease and health management; (6) consider ecological, biological, and life-history patterns when forming enhancement objectives and tactics; (7) identify released hatchery fish and assess stocking effects; (8) use an empirical process for defining optimum release strategies; (9) identify economic and policy guidelines; and (10) use adaptive management. Developing case studies with Atlantic cod *Gadus morhua*, red drum *Sciaenops ocellatus*, striped *Mugil cephalus*, and white seabass *Atractoscion nobilis* are used to verify that the responsible approach to marine stock enhancement is practical and can work.
Descriptors: marine fish, fishery development, cultured organisms, stock assessment, resource management, population genetics, stocking density, *Gadus morhua, Sciaenops ocellatus, Mugil cephalus, Atractoscion nobilis*, Gadidae, Mugilidae, Sciaenidae, USA coasts
ASFA; Copyright © 2003, FAO

Brennan NP, DeBruler R, Blankenship HL, Leber KM (2001) **Coded-wire tag and visible implant elastomer tag retention in juvenile red snapper *Lutjanus campechanus*.** *Aquaculture 2001: Book of Abstracts.* p. 77
As part of the Gulf of Mexico Marine Stock Enhancement Program, a series of tag retention experiments on juvenile red snapper, Lutjanus campechanus, reared at the Gulf Coast Research Laboratory in Ocean Springs, MS were initiated to adapt current tagging technology to red snapper stock enhancement research. Coded-wire tags (CWT) and internal visible implant elastomers (VIE) (NMT, Shaw Island, WA, USA) were tested in different body locations of two size classes (50-80 mm, and 70-110 mm SL). Juveniles were anesthetized and weights and lengths were taken from subsamples of the populations tagged. CWTs were then injected free-hand with a Mark IV tagging machine into the nose cartilage (n=35), the left cheek muscle (n=35), the nape muscle (n=40), the posterior dorsal muscle (n=35), and the ventral caudal peduncle muscle (n=70). Initial tag presence was verified with a magnetic field detector. VIE material was also injected with hand pressurized hypodermic syringes anteriorly and diagonally across the nose bridge (n=40), under the skin at the base of the anal fin (n=35), and under the skin of the ventral caudal peduncle muscle (n=35). Due to

a shortage of fish 2 CWTs and 1 VIE mark was implanted in each fish in different combinations. Tag retention checks were performed at 3 weeks and 6 weeks after tagging. CWT retention rates had stabilized 3 weeks after tagging, by 6 weeks CWT retention remained at 100% in the nape and cheek muscle, followed by the nose cartilage (97%), the caudal peduncle muscle (96%) and the posterior dorsal muscle (90%). Because of the excellent retention rates of the CWT's in the nape muscle, and because the CWT's could most easily be injected at this site, the nape muscle was selected as the CWT target site for future stock enhancement experiments. Development of head molds would facilitate faster tagging and accurate tag placement and efforts are underway to develop these. VIE retention was also excellent; 100% of the snapper retained VIE material in the nose bridge, and anal fin muscle, and 95% retained VIE material in the caudal peduncle at 6 weeks. Although VIE material was initially quite visible, 3 weeks later VIE marks in the caudal peduncle, and anal fin muscle were difficult to see under natural lighting conditions due to pigmentation over the VIE. Although the nose bridge site promised high retention and the best visibility in this study additional studies are being performed with VIE material of different colors in the caudal fin rays and other body locations for improved visibility. There was no correlation between the fish sizes tested and tag retention.
Descriptors: marine aquaculture, fish culture, tags, tagging, juveniles, stocking (organisms), stock identification, hatcheries, aquaculture techniques, marine fish, *Lutjanus campechanus*, ASW, Mexico Gulf, tag retention, visual implant elastomers, red snapper
ASFA; Copyright © 2003, FAO

Bruyndoncx L, Knaepkens G, Meeus W, Bervoets L, Eens M (2002) **The evaluation of passive integrated transponder (PIT) tags and visible implant elastomer (VIE) marks as new marking techniques for the bullhead.** *Journal of Fish Biology.* 60(1):260-26
NAL Call No. QL614 J68
To test the reliability of PIT tags and VIE marks as new marking techniques for the bullhead *Cottus gobio*, different tagging treatments were assayed. The relatively high recapture rates suggest the applicability of both marking techniques for this small benthic fish species. Copyright 2002 The Fisheries Society of the British Isles
Descriptors: tracking, local movements, freshwater fish, tagging, marking and tracking techniques, *Cottus gobio*, bullhead
ASFA; Copyright © 2003, FAO

Close TL, Jones TS (2002) **Detection of visible implant elastomer in fingerling and yearling rainbow trout.** *North American Journal of Fisheries Management.* 22(3):961-964
NAL Call No. SH219.N66
Visible implant elastomer (VIE) was evaluated for marking yearling rainbow trout *Oncorhynchus mykiss*. The rate of tag detection after the yearlings had been at large for 35 months was also compared with the detection rate found in a subsequent year-class that had been marked as fingerlings and had been at large for 29 months. Fish were marked in the postocular adipose eyelid tissue and on the ventral surface of the lower jaw and examined in the dark under ultraviolet light. Detection rates for both year-classes ranged from 29% to 33%, based on the proportion of fish with two detectable marks. Because the marks were not easily recognized, we believe the detection rates were overestimated. We conclude that detection of VIE at the sites we chose was problematic for the strain of rainbow trout we marked. More favorable long-term detection rates in other species suggest that poor detection rates may be unique to heavily pigmented strains of rainbow trout.
Descriptors: marking, tracking, tagging, freshwater fish, fishery management, *Oncorhynchus mykiss*, rainbow trout
ASFA; Copyright © 2003, FAO

Knights BC, Lasee BA (1996) **Effects of implanted transmitters on adult bluegills at two temperatures.** *Transactions of the American Fisheries Society.* 125(3):440-449
NAL Call No. 414.9 AM3
Laterally compressed panfishes are small and have limited intraperitoneal space; thus, they may suffer adversely from surgically implanted transmitters even if the transmitter meets the generally recommended ratio of transmitter weight to fish weight of 2%. We studied the effects of intraperitoneal transmitters (2.81 g) on survival, growth, healing, and health of bluegills *Lepomis macrochirus* (mean weight 133 g) held for 8 weeks at 6°C and 20°C. Radio-tagged bluegills at 20°C had a mortality rate of 10% and tag loss rate of 15%. At 6°C, bluegills had no mortality or tag loss. Radio-tagged and reference fish fed in both 20°C raceways; however, a few reference fish appeared dominant at feeding time. This dominance by a few reference fish was also indicated by a large weight gain for three reference fish in each 20°C raceway. At 6°C, neither reference fish nor radio-tagged fish fed activity. Radio-tagged fish held at 20°C exhibited pelvic fin erosion, erythema and necrosis at the antenna exit and at suture insertions, and lost or loose sutures, effects not observed in other test fishes. Examination of fish held at 20°C also showed enclosure of the transmitters in a fibrous capsule and adhesion of visceral organs. Epithelialization over the incision occurred in radio-tagged bluegills at both temperatures, but there was little further healing at 6°C. At 20°C, tissue responses included chronic inflammation and dermal granulation. Radio-tagged fish did not appear to be more susceptible than reference fish to bacterial infection. Mortality, adverse morphological effects, altered behavior, and limited healing in bluegills suggest that implanted transmitters impaired their health. Thus, movement and habitat use data collected by telemetry for this species and perhaps for other panfishes should be interpreted with caution.
Descriptors: tagging mortality, tags, *Lepomis macrochirus*, sonic tags, biotelemetry, biological stress, mortality, stress
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Mahapatra KD, Gjerde B, Reddy PVG, Sahoo M, Jana RK, Saha JN, Rye M (2001) **Tagging: on the use of passive integrated transponder (PIT) tags for the identification of fish.** *Aquaculture Research.* 32(1):47-50
NAL Call No. SH1 F8
To determine the efficacy of passive integrated transponder (PIT) tags for marking rohu *Labeo rohita* (Ham.) in the selective breeding programme, a series of experiments has been carried out at the Central Institute of Freshwater Aquaculture (CIFA) under the Indo-Norwegian project of 'Selective breeding of rohu'. Six groups of rohu fingerlings with weight ranging from 2 g to 20 g were tagged with PIT tags to determine a suitable size range for tagging. Fingerlings weighing 8-15 g were found to be quite suitable for tagging with a PIT tag. Recovery of the PIT tag depends upon the survival of tagged fish under field conditions. Rejection of the PIT tag by rohu was observed to be only 0.05%. Through effective management practice, the survival of tagged fish increased up to 95%, and thus tag loss was minimized.
Descriptors: tagging, identification, acoustic transponders, induced breeding, fish culture, fishery management, *Labeo rohita*
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Mangan BP (1998) **Long-term retention of a radio transmitter by a muskellunge.** *Journal of Freshwater Ecology.* 13(4):485-487

NAL Call No. QH541.5.F7J68
The use of surgically implanted radio transmitters in fish is widespread. There are, however, some questions concerning retention time of transmitters and effects on fish health. The author serendipitously recovered a large adult muskellunge implanted with a radio transmitter for 13 years. Although a large fibrous mass was associated with the transmitter, this ripe female otherwise appeared to be disease-free.
Descriptors: tags, tagging mortality, biotelemetry, tracking, radio telemetry, Esox masquinongy
ASFA; Copyright © 2003, FAO

Martin SW, Long JA, Pearsons TN (1995) **Comparison of survival, gonad development, and growth between rainbow trout with and without surgically implanted dummy radio transmitters.** *North American Journal of Fisheries Management.* 15(2):494-498
NAL Call No. SH219.N66
The use of radio telemetry to determine fish movement patterns associated with spawning has proliferated in recent years. However, little is known about the effect of surgically implanted radio transmitters on spawning behavior or gonad development of fish collected near the time of spawning. We compared survival, gonad development, and growth between wild rainbow trout *Oncorhynchus mykiss* with and without dummy radio transmitters that were surgically implanted prior to the fish's spawning period. Wild rainbow trout (mean fork length, 351 mm) were collected from the Yakima River, Washington, on February 5, 1993, with a drift boat electrofisher. Ten fish that met selection criteria had dummy radio transmitters surgically implanted into the intraperitoneal cavity, and ten other fish were retained as controls. All 20 fish were released into a nearby pond and fed daily. After 47 d all fish were measured and weighed, and gonad development and general health were assessed. All fish survived, and there were no transmitter expulsions by treatment fish. There were no significant differences in weight, condition factor, or gonad development between treatment fish and control fish. These results suggest that wild rainbow trout may be used for telemetry studies, even when the collection of fish and transmitter implantation occurs close to the time of spawning.
Descriptors: *Oncorhynchus mykiss*, tagging mortality, biological development, growth, biotelemetry, USA, Washington, Yakima R., sexual maturity, salmon fisheries, fishery management, anadromous species, telemetry
ASFA; Copyright © 2003, FAO

Olsen RE, Henderson RJ (1997) **Muscle fatty acid composition and oxidative stress indices of Arctic charr, *Salvelinus alpinus* (L.), in relation to dietary polyunsaturated fatty acid levels and temperature.** *Aquaculture Nutrition.* 3(4):227-238
NAL Call No. SH156.A658
The influence of feeding high levels of polyunsaturated fatty acids (PUFA) on muscle fatty acid composition and indices of oxidative damage was examined in Arctic charr, *Salvelinus alpinus* (L.). All diets contained 100 g/kg lipid of dry weight. Two diets contained marine fish oils giving a PUFA level of 250 g/kg and 500 g/kg of lipid. The remaining two diets contained vegetable oils high in either 18:2n-6 or 18:3n-3, giving a PUFA level of more than 500 g/kg of dietary lipid. The charr were maintained at 8°C until their weight doubled, and were then transferred to 0.8°C for 30 days. Growth was similar in all groups. The fatty acid compositions of muscle were influenced by dietary PUFA but were less diverse than those of the diets. The overall pattern of fatty acid compositions indicated preferential desaturation and elongation of n-3 PUFA coupled with selective oxidation of 18:2n-6. Total n-3 PUFA

content in TAG was always lowered compared with the diet, suggesting a specific mechanism for the removal of these fatty acids. Subjecting the fish to low temperature increased PUFA content in muscle of charr fed the 250 g/kg marine n-3 PUFA diet, but had no effect on the other treatments. For fish at 8°C, no significant differences were found between groups in terms of haematocrit, plasma alanine aminotransferase (ALAT), and plasma and muscle thiobarbituric acid reactive substances (TBARS), although there was a tendency towards increased levels of TBARS in the group receiving 500 g/kg marine n-3 PUFA of lipid. Subjecting the muscle to forced oxidative conditions resulted in increases in TBARS in all groups, particularly those fed 500 g/kg marine n-3 PUFA. Lowering the environmental temperature corresponded with a further increase in the plasma ALAT and muscle TBARS in this group. It is concluded that feeding diets containing high levels of long-chain n-3 PUFA may be detrimental to the fish's health and flesh quality, particularly at low environmental temperatures.
Descriptors: muscles, fatty acids, biochemical composition, food conversion, nutritional requirements, temperature effects, biological stress, *Salvelinus alpinus*, diets, growth, Arctic char

Petry H (1976) **Experiments for the registration of fish toxicants.** *The influence of environmental factors upon the health of fishes. Die Einwirkung von Umweltfaktoren auf die Gesunderhaltung des Fisches*. No. 2. (In German with English summary)
A quantified registration of spontaneous activity of animals can be carried out easily and in a reliable way using the principle of magnetic induction. To study the practicability of this method for continuous control of waters using test fish as indicators, changes of spontaneous activity of magnet marked trouts exposed to detergents have been detected by induced voltages.
Descriptors: bioassays, methodology, tagging, magnetism, *Oncorhynchus mykiss*, Pisces

Pollard MJ, Kingsford MJ, Battaglene SC (1999) **Chemical marking of juvenile snapper, *Pagrus auratus* (Sparidae), by incorporation of strontium into dorsal spines.** *Fishery Bulletin*. 9(1):118-131
NAL Call No. 157.5 B87
The aim of this project was to investigate the use of strontium as a chemical tag in the dorsal spines of the marine teleost *Pagrus auratus* that would allow the mass tagging of juvenile fish. Previous studies in which the incorporation of strontium has been experimentally manipulated for the purposes of marking have generally concentrated on freshwater and anadromous species. This is the first study to investigate the tagging of spines with strontium, the removal of which is non-destructive. Inductively coupled plasmamass spectrometry (ICP-MS) was used to measure isotopic concentrations. The dorsal spines of juvenile *P. auratus* that had been immersed in salt water containing 0.125 g/L $SrCl_2$ x $6H_2O$ (5x ambient strontium) and 0.250 g/L (10x ambient) for five days incorporated ^{86}Sr at levels greater than those in control fish. The strontium signal was persistent in spines for at least 36 days and showed no sign of decay during the experiment. No effects of the treatments on fish health or growth were detected. Short-term immersion experiments (6 hours to 5 days) indicated that treatments of 10x ambient or greater for 4-5 days were required to tag fish reliably with strontium. Natural levels of strontium in the spines of juveniles varied among locations separated by tens of kilometres along the coast of New South Wales. Natural variations in strontium concentrations were not great enough, however, to obscure the differences between

tagged and wild fish. It was concluded that strontium immersion is a useful and relatively environmentally safe method of tagging large numbers of small fish.
Descriptors: tagging, strontium, dorsal fins, marking, fins, *Pagrus auratus*, PSE, Australia, New South Wales
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Schweigert J, Flostrand L, Slotte A, Tallman D (date not provided) **Application of coded wire tagging technology in Pacific herring to investigate stock structure and migration.** *International Council for the Exploration of the Sea, Palaegade 2-4 DK-1261 Copenhagen Denmark. [vp].*
Tagging of Pacific herring in British Columbia to understand stock structure and mixing rates of populations using internal metal tags and external Floy tags has a long history dating to the mid-1930s. Unfortunately, uncertainty in some tag recovery locations and low rates of tag return limited the utility of these studies. In 1999, a new tagging program was initiated employing coded wire microtags to mark Pacific herring on the spawning grounds to monitor the movement and mixture of fish interannually. Tank experiments indicated high rates of survival and low tag shedding rates, and field trials indicated the feasibility of cost effective application of large numbers of tags during the short spawning season (250,000 tags applied over 28 days). Methodologies for capturing, holding, tagging, and releasing tagged herring were developed. Tank experiments also investigated the effects of the location of tag insertion and anaesthetic on short-term (3 months) survival and tag retention. Tag detection tubes designed for recovery of tagged Pacific salmon were adapted to detect and recover Pacific herring in fish plants during roe extraction processing. Tag recovery rates of 1-2% in 2000 from the 1999 releases greatly exceeded the returns from previous tagging programs. Tag returns indicated a high degree of homing or fidelity to the area of release, but also produced a number of remarkable strays. Coded wire tagging technology appears to provide a useful tool for large-scale marking experiments on smaller pelagic species and should have broad application for stock structure and mark-recapture studies.
Descriptors: tagging, tags, survival, fish handling, migrations, clupeoid fisheries, stock identification, *Clupea pallasi*, INE, Canada, British Columbia, coded wire tags, Pacific herring
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Stobo WT (1972) **The effect of dart tags on yellow perch.** *Transactions of the American Fisheries Society.* 101(2):365-366
NAL Call No. 414.9 AM3
Yellow perch carrying T-bar spaghetti tags in heavily vegetated areas showed wounds around the point of insertion, which might cause differential mortality in comparison with untagged fish. The retention and possible effects of the tags are investigated. 46 perch seined from the Ottawa River were tagged with FD-67 dart tags using Dell's method but without anaesthetic. They were then released back into the river. In the lab 16 perch were tagged plus and minus anaesthetic and/or alcohol treatment for the equipment. 26 specimens recaptured form the field showed no tag loss; open wounds occurred at the point of attachment, but without apparent infection. In the lab wounds were smaller but otherwise similar. It was concluded that the field tagging procedure was not responsible for the occurrence of the wounds and it is therefore satisfactory. Tag retention is good and mortality insignificant.
Descriptors: yellow perch, FD-67, field tagging procedure
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Thoreau X, Baras E (1997) **Evaluation of surgery procedures for implanting telemetry transmitters into the body cavity of tilapia *Oreochromis aureus*.** *Aquatic living resources/Ressources vivantes aquatiques. Nantes [Aquat. Living Resour./Ressour. Vivantes Aquat.]* 10 (4):207-211.

NAL Call No. SH1.A8

Surgery procedures were used to implant telemetry transmitters into the body cavity of adult (574 - 1033 g) tilapias *Oreochromis aureus* in aquaculture tanks (4 m^2,1.5 m^3, 26.5 ± 0.5°C, greater than or equal to 5.0 mg O_2/L) and their effect on fish survival, growth and behaviour was evaluated. Only one out of 35 implanted fish died. With one exception, all fish consistently retained their transmitter until the end of the study (up to 30 months). Healing was faster when the incision was sutured with polyamide monofilament (5-14 days) than with other suture materials, due to tunnelling with atraumatic needles for catgut or fouling of braided silk. In all 10 fish sacrificed after 30 and 50 days, the transmitter had become encapsulated by connective tissue. No infection or damage to the viscera was observed. The activity of four tilapias (903-1033 g) equipped with motion sensitive transmitters was telemetered during the recovery from anaesthesia and surgical procedures. All four fish maintained a normal diurnal activity rhythm pattern throughout the study but had low levels of activity during the first 12-24 h. Based on the evolution of their resting posture after surgery, it is suggested that tilapias need 3 to 4 days to completely compensate the negative buoyancy resulting from anaesthesia and tagging.

Descriptors: telemetry, tagging, aquaculture, tanks, warm-water aquaculture, *Oreochromis aureus*, Pisces

Turner SE, Proctor GW, Parker RL (1974) **Rapid marking of rainbow trout.** *Progressive Fish Culturist.* 36(3):172-174

NAL Call No. 157.5 P94

The authors describe the apparatus used when cold branding 428,895 rainbow trout prior to release in Lake Taneycomo, Missouri. After the fish had been anaesthetised with methypentynol they were placed against the silver brand which was cooled by liquid N_2. One marking table could brand {approx} 200 trout per hr. Mortality was between 0.05 and 1.80% for branded trout, mostly as a result of handling or the anaesthetic. Most marks were identifiable, even after an increase in size from 4.0 to 24.0 in. The most legible marks were I, X and O. Mark durability on trout >10 in total length was not judged but it is noted that normal abrasions found on hatchery trout of this size would make it difficult to identify brands of the size used on the 4-6 in fish.

Descriptors: marking, *Oncorhynchus mykiss*

Web Resources:

Chapter 7 Fish Welfare and Health in Relation to Tagging, written by Prof. John Davenport (group leader), Dr. Etienne Baras, Dr. Gianna Fabi, and Dr. Gisli Jonsson,
http://www.hafro.is/catag/g-reference/chapter_7.html

excerpted from **Thorsteinsson V (2002) Tagging Methods for Stock Assessment and Research in Fisheries, Report of Concerted Action FAIR CT.96.1394 (CATAG). Reykjavik. Marine Research Institute Technical Report (79), pp 179**
http://www.hafro.is/Timarit/catag.pdf

The Welfare of Fish and Aquatic Invertebrates
Submission of the Australian and New Zealand Federation of Animal Societies
September 1992
http://www.Pisces.demon.co.uk/welfare.html

3.3. REGULATORY ISSUES

Allan G, Heasman H. (2001) **The ethics of research with fish: who is watching the scientists and why?** *Fish NSW*. 4(1):40-41

In New South Wales (NSW) (Australia), the Animal Research Act of 1985 was introduced to protect the welfare of animals and it applies to any organization or individual that uses or supplies vertebrate animals for research or teaching in NSW. NSW Fisheries conducts research with fish to help achieve the corporate goals and all research involves some handling of fish. Where fish surveys or tagging are undertaken, some fish may be killed but most fish are returned unharmed to the environment. In aquaculture research, the overwhelming emphasis is on keeping fish in the best possible, low stress conditions in order to promote rapid growth and natural reproduction. In all cases, the ultimate goal is to protect and manage fisheries resources for the benefit of current and future generations. The establishment and maintenance of the NSW Fisheries Animal Care and Ethics Committee, under the Animal Welfare Act, helps to ensure that scientists consider the ethics of the research they conduct and that these ethics accord with the views of the community. Of equal importance is the need to reassure the community that ethics are given prime consideration before any animal experimentation commences.

Descriptors: fish, aquaculture enterprises, research programmes, research institutions, Australia, New South Wales

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Animal and Plant Health Inspection Service, Hyattsville, MD. (2002) **Licensing and Registration Under the Animal Welfare Act. Guidelines for Dealers, Exhibitors, Transporters, and Researcher.** Corporate Source Codes: 061345000. *Report Number: USDA/APHIS-Program Aid-1117.* 24p

Note: Order this product from NTIS by: phone at 1-800-553-NTIS (U.S. customers); (703)605-6000 (other countries); fax at (703)605-6900; and email at orders@ntis.gov

NAL Call No. 1 Ag84Pro no.1117

Many businesses that buy or sell warmblooded animals, exhibit them to the public, transport them commercially, or use them in experiments or teaching must be licensed or registered by the U.S. Department of Agriculture (USDA). Normal farm-type operations that raise, or buy and sell, animals only for food and fiber, and businesses that use only fish and other coldblooded animals are exempt by law; those that use only rats, mice, or birds are exempt by regulation. The rabbit business is exempt from regulation if the rabbits are intended for food or fiber. If any rabbits are designated for use I n the pet, exhibit or laboratory-animal trade, the business is regulated. Certain other types of businesses are specifically exempt by law or regulation. No exempt business has to be licensed or registered.

Descriptors: animal husbandry, licensing, guidelines, permits, commerce, homeotherms, law (jurisprudence), legislation, federal law, businesses, regulations, standards, inspections, APHIS, animal welfare

Brooman S, Legge D (2000) **Animal welfare vs free trade--free trade wins: an examination of the animal welfare implications of R v Ministry of Agriculture, Fisheries and Food ex p Compassion in World Farming (1998).** *Animal Welfare.* 9 (1):81-85.

NAL Call No. HV4701.A557

Descriptors: European Union, animal welfare, social legislation, free trade, veal calves, calf housing, fish

Brosse H, Wilmsmann F (1994) **Zur Verfassungsmassigkeit 'tierschutzerischer' Bestimmungen im Landesfischereirecht. [The constitutionality of 'animal welfare' provisions in fishery legislation of the Lander.]** *Agrarrecht* 24(10):323-325 (In German)
ISSN: 0340-840X
Under the German constitution the Lander are empowered to legislate to preserve and control inland fisheries, including angling. Detailed examination of their powers in this field and in that of animal welfare leads to the conclusion that legislation forbidding the use of live fish as bait or the holding of live fish in catch nets is within the legal competence of the Lander under the constitution.
Descriptors: angling, legislation, fisheries, animal welfare

Burgoyne D (1999) **International trade and animal welfare.** *Cahiers Agricultures.* 8(6):445-449 (In French with English summary)
NAL Call No. S5.C34
The World Trade Organization (WTO), the successor to GATT, has an increasingly important role in the way agricultural commerce is conducted between countries and within our own borders. The last GATT agreement signed in 1994 reflects the concerns of the majority of the member countries with regard to the health protection of plants, animals and humans for agricultural and agrifood trade. Since WTO's mandate took effect on 1 January 1995, agricultural and agrifood trade between member countries must respect its rules. There are basically three places under which an animal welfare protection measure could fall: Article XX of the General Agreement, the Agreement on the Application of Sanitary and Phytosanitary (SPS) Measures and the Agreement on Technical Barriers to Trade (TBT). Article XX of the General Agreement permits a member country to, among other actions, take the measures it views necessary to protect public moral values. However, these measures must respect the Agreement on the Application of SPS Measures and the Agreement on TBT. To date, no member country has called on these exemptions to restrict imports of agricultural products. At this point in time, we do not clearly know how this clause could ensure the respect of animal welfare because there are no precedents on the subject. During the Uruguay Round, the European Union wanted to introduce other measures into the Agreement on the Application of SPS Measures. To assess the legitimacy of an SPS measure, the EU promoted such other considerations as: the possibility of deciding which preferences the majority of consumers hold, the existence of environmental standards in the country of origin with respect to production methods, as well as the level of animal welfare in the country of origin of the animal. These latter two measures were not adopted because they would lead to sovereignty infringements between countries. A compulsory measure that would dictate how an animal should be kept, fed and transported in its country of origin before being imported would introduce an oversight over the laws of another country. Such an example presented itself recently. In October 1998, Switzerland officially notified the WTO members that it was considering adopting an SPS measure that would have forced the labeling of imported eggs produced by hens kept in cages banned in its country. Canada, along with a number of other countries, asked Switzerland to explain the reasons behind that measure. Could it justify what the measure was aimed at protecting? Could it identify the risk that eggs from hens kept in battery cages elsewhere in the world would present for animals and humans in Switzerland? In December 1998, the Swiss government removed all reference

to the method of production of imported eggs from its final regulation. Notwithstanding this example, a country may adopt measures consistent with its WTO obligations to prevent stressed, injured, dehydrated or sick animals from entering its territory. The difficulty in creating international rules on animal welfare stems from the fact that members cannot agree on a common definition of intangible concepts like stress and of its methods of analysis to come up with an objective evaluation. In the agri-food sector, TBT or normative measures, refer mostly to technical regulations and voluntary standards. For products such as meat, it would be difficult to determine whether or not it is from an animal that was raised with or without cruelty when there are only laboratory analyses to rely on. This is why a compulsory technical rule on the importation of meat and related products which aims to ensure the respect of animal welfare will be contested. A member country can nonetheless take certain measures with respect to animal welfare while respecting its obligations towards other members. All countries can freely adopt standards and regulations aimed at domestic animal welfare. A direct subsidy scheme can, in that case, best solve the problem of inequality of production costs. However, all taxpayers end up supporting this production method. Secondly, for imported products, it is possible to adopt a voluntary system of labeling with the aim of identifying products that respect animal welfare. For example, the WTO ruling in the case of the effects of tuna fishing on dolphins said that positive labeling was a legitimate measure to limit the use of the "dolphin-friendly" label solely to products that respect this fishing standard. The organic food sector currently uses such a system with success. The advantage to the taxpayer is that the consumer who chooses to buy food produced under these norms is also financially supporting the system. Lastly, it is also possible for countries with kindered spirits to facilitate the trade of products that meet certain animal welfare standards by negotiating mutual recognition agreements-provided that they do not restrict trade from other countries.
Descriptors: animal husbandry (agriculture), government and law, agrifood industry, European Union, World Trade Organization {WTO}, GATT successor, application of sanitary and phytosanitary measures {SPS Measures}, agricultural commerce, animal welfare, international agreements, international regulations, international trade, negotiations, trade regulation, voluntary standards

Cook, R.H., Simpson, F.J. (1995) **Roles of government agencies in aquaculture development in Atlantic Canada: Regulation and incentives.** (Ed:) Boghen AD. *Cold Water Aquaculture in Atlantic Canada, 2nd Edition.* Univ. Moncton, NB, Canada Cirrd. pp. 503-538
NAL Call No. SH37.5 A8C64 1995
Aquaculture began in Canada under existing federal, provincial, and municipal acts designed for the regulation of a wide range of human activities. The municipalities established and managed zoning regulations, licensed businesses, and assessed property taxes. The provinces administered laws respecting property and civil rights, the enhancement and control of natural resources and the environment, and the promotion and control of provincial commerce, education, and health and welfare within their boundaries. And the federal government was responsible for the regulation, conservation, and protection of the fishery and sponsored most of the research and development work in support of the industry. Some of the federal government's regulations concerning the practice of agriculture (e.g., Pest Control Products Act) also apply to aquaculture, as do those pertaining to protecting navigable waters, the environment and fish habitat, health and product safety, control of

introductions and transfers of fish, interprovincial and international trade, and the administration of criminal law, as well as all other matters that affect Canadians as a whole.
Descriptors: aquaculture regulations, Canada, governments, aquaculture development, environmental protection, resource conservation, aquaculture enterprises, animal welfare

Costello MJ, Grant A, Davies IM, Cecchini S, Papoutsoglou S, Quigley D, Saroglia M. (2001) **The control of chemicals used in aquaculture in Europe.** *Journal of Applied Ichthyology.* 17(4):173-180
NAL Call No. QL614 Z44
A range of chemicals are used in European marine aquaculture and these may be categorized as disinfectants, antifoulants and medicines (includes vaccines). This article provides a review of chemicals used in aquaculture in Europe, their regulatory status, and a checklist of points considered best practice in the use (and avoidance of use) of medicines in marine aquaculture. The release of antifoulants and disinfectants into the marine environment is controlled by local and/or national waste discharge regulations that may in turn be guided by wider environmental quality objectives. The authorization of veterinary medicines, biologicals (vaccines) and pharmaceuticals (chemicals), in Europe is the subject of several EC Directives. Registration dossiers address the issues of product quality, safety and efficacy and include environmental and consumer safety where the product is destined for use in a food-producing animal. Fish farmers, like all livestock producers, must have access to a range of properly authorized medicines to safeguard animal health and welfare. The distribution and supply of medicines must be appropriately controlled and their authorization appropriately includes environmental risk assessment to a common European Union (EU) or international standard. There is progress towards the harmonization of the authorization process within the EU and this will help to ensure the continued availability of medicines for fish. Consumer safety is addressed by the setting of maximum residue limits (MRLs) derived through toxicological risk assessment and by surveillance of food for residues of veterinary medicines. The system for the environmental risk assessment of chemicals used in aquaculture is being developed and is outlined in the present article. It is recommended that the supply and use of fish medicines is uniformly regulated in the EU and supported by appropriate codes of best practice. A number of codes of practice that include reference to the use of medicines have been produced both at a European level and in member states. It is recommended that all European marine aquaculture producers adopt a code of best practice for the use of medicinal and other chemicals their industry. Medicines are one part of an integrated package in dealing with animal health. This includes environmental conditions, nutrition and hygiene. The best practice guidelines presented here are based on the outcome of three European workshops as part of the EU MARAQUA project that involved industry, government and research scientists. They cover the avoidance and minimizing of the need to use medicines and other chemicals, to recording and monitoring their use and effectiveness (in case of resistance development), exchange of experiences within the industry, and staff training. Recommendations are also included for manufacturers of medicines and other chemicals, and for regulatory authorities. Minimizing the need to use medicines and other chemicals requires attention to a healthy source of fish stock. Staff must be appropriately trained in fish husbandry (to minimize stress), hygiene and disease recognition and treatment, including management of the farm site to keep it disease free. The latter may require single generations of fish per site to allow a fallow period during which a disease or parasite cycle is broken. These recommendations and guidelines are in accordance with the current codes of practice being developed by different sectors of the aquaculture industry in different

countries. They do not necessarily involve significantly higher production costs and indeed are more likely to save costs as medicines and disease impacts are very costly to industry.
Descriptors: marine aquaculture, medicine, disinfectants, antifouling substances, waste disposal, legal aspects, *Salmo salar, Oncorhynchus mykiss, Dicentrarchus labrax, Sparus aurata*

Damsgaard B (1997) **Fiskens velferd - Kan vi forene en etisk riktig produksjon og en konkurransdyktig naering?[The welfare of the fish - can we combine an ethic correct production with a competitive business?].** *Norsk Fiskeoppdrett, Naeringsliv- Politikk Marked Forsknin.* 22(4):30-31,33 (In Norwegian)
ISSN: 0332-7132
Descriptors: fishes, fish culture, animal welfare, behaviour, stress, ethics, aquaculture

Frohlich T, Steinigeweg W (1995) **Tierschutzrechtliche Uberwachung von Tierborsen, ein amtstierarztliches Stiefkind? [Supervision of animal welfare regulations in animal markets--an official veterinary stepchild?].** *Deutsche tierarztliche Wochenschrift* (Germany). 102(3):131-133. (In German with English summary)
NAL Call No. 41.8 D482
The authors report about problems in animal welfare during commercial animal exhibitions. A list of claims in accordance to animal welfare for these forms of animal presentation is given. Remaining problems are described.
Descriptors: animal welfare, legislation and jurisprudence, domestic, commerce, veterinary medicine, commerce, standard, fishes, Germany, poultry

Keresztessy K (1993) **A magyar halfajok vedettsegenek uj szabalyozasa. [New animal welfare regulations on protection of fish species in Hungary.]** *Halaszat.* 86(3):114-116 (In Hungarian)
NAL Call No. 414.8 H12
Descriptors: legislation, freshwater fishes, animal welfare

Knierim U (1996) **Die Tierscutz-Schlachtverordnung. [The animal welfare regulations at slaughter.]** *Deutsche Tierarztliche Wochenschrift* 103(2):52-54 (In German with English summary)
NAL Call No. 41.8 D482
The animal welfare regulation on the slaughter of animals, existing only as a draft for the time being, is designed not only to transpose EC-legislation into national law but also to update and strengthen preconstitutional national legislation on this matter. For a wide area related to the slaughter or killing of animals, animal welfare requirements are put in concrete terms. Among the topics belonging to this area are the theoretical and practical knowledge of the personnel, the handling of animals before slaughter or killing, stunning, the control of its efficacy and the permessibility of certain stunning or killing methods. Not only livestock but also, for example, fur animals and fish are concerned. In practice it will take some efforts in order to attain compliance with the provisions of the animal welfare slaughter regulation.
Descriptors: regulations, stunning, poultry, legislation, slaughter, animal welfare, cattle, pigs, sheep, fishes

Koenigs E (1988) **Tierschutzaspekte im Fischereirecht[Animal protection aspects in legal fishery].** *Deutsche Tieraerztliche Wochenschrift*. 95(2):58-60. (In German with English summary)
NAL Call No. 41.8 D482
Descriptors: fishes, fisheries, legal principles, animal welfare, animal health, animals, aquatic animals, aquatic organisms, economic sectors, fishing industry, legislation

Lahteensmaki V (1987) **Legislation dealing with animal experimentation in Finland**. *Animal Technology*. 38:229-233.
NAL Call No. QL55.I5
Descriptors: fish, animal welfare, legal principles, legislation, animal health, research

Nowak D (1993) **Tierschutzrelevante Probleme bei der Kontrolle von Zoofachgeschaften aus amtstierarztlicher Sicht [Problems relevant to animal welfare in the control of pet shops from the viewpoint of official veterinarians].** *Wochenschrift* (Germany). 100(2):76-68. (In German with English summary)
NAL Call No. 41.8 D482
Results of an inquiry of all official veterinarians in Berlin are presented, concerning the situation of animal welfare in pet shops. Typical problems of keeping animals in pet shops are revealed. Many of the official veterinarians make complaints about the lack of practical guidelines, manuals or expert opinions when they are asked to give legal assessments. Existing recommendations, e.g. for cage sizes, are of a broad variety, which is underlined by some demonstrative pictures.
Descriptors: animal husbandry, standard, animal welfare, domestic, commercial standards, Berlin, birds, cats, dogs, fishes, housing, standards, reptiles, rodentia

Olson KR, Crawford RL, Gingerich WH, Meyerhoff RD, Miller JG (1991) **The Animal Welfare Act: Implication and predictions in lower vertebrate research.** 14. ASTM Symp. on Aquatic Toxicology and Risk Assessment, San Francisco, CA (USA), 22-24 Apr 1990. Aquatic Toxicology and Risk Assessment: Fourteenth Volume. Eds: Mayes, MA, Barron MG. Philadelphia, PA USA American Society for Testing and Materials. pp. 5-11.
NAL Call No. QH545 W3S95
The use of animals in basic and applied research is governed by both scientific objectives and ethical considerations. Heightened concern over the humane treatment of experimental animals by the scientific community and the public has led to the development of guidelines and regulations concerning animal care and use. Most of these regulations, to a large extent under the purview of the U.S. Department of Agriculture (USDA) and federal granting agencies such as the National Institutes of Health (NIH), are directed towards research on higher vertebrates, especially mammals. Guidelines for care and use of lower vertebrates are minimal to nonexistent and are frequently extrapolated from mammalian models. However, it appears that further regulation of lower vertebrate research is probable. This paper summarizes a panel discussion that was conduced at the 14th ASTM Aquatic Toxicology Symposium on 22 April 1990. The panel consisted of two members from federal regulatory agencies (USDA and NIH) and three members from research institutions (representing industrial, Federal, and academic laboratories). The purpose of this discussion was to establish a dialogue on the care and use of lower vertebrates in research. Included is a discussion of the history and future of animal welfare legislation and how the various regulations are implemented in the three laboratory situations. Emphasis will be on fish, although the principles of animal care and use could apply to other ectotherms as well. A

number of key issues need to be addressed by the scientific community relative to lower vertebrate research: (1) Should additional guidelines be established? (2) Who should establish them? (3) What is the role of the investigator in formulating guidelines that will affect his/her research? (4) Are separate guidelines needed for different organizations (university, industry, and government) and for specific applications such as animal holding-maintenance versus experimentation? (5) Should general or specific guidelines be identified? (6) What criteria should be used? (7) How can this be implemented? The consensus of the panel is that it is in the best interest of the investigators to take an active role in the development of suitable guidelines for the animals with which they work. Possible mechanisms to achieve this are discussed.
Descriptors: environmental legislation, environmental monitoring, bioassays, pollution indicators, indicator species, test organisms, legislation, USA, government policy, toxicity testing

Pritchard DG (2001) **The role of the Ministry of Agriculture, Fisheries and Food in animal welfare.** *Research in Veterinary Science.* 70(Supplement A):53
NAL Call No. 41.8 R312
Descriptors: animal care, government and law, fisheries, animal welfare

Sauer N, Manz D (1994) **Tierschutztatbestande bei Fischen. [Animal welfare legislation and fish.]** *Tierarztliche Umschau.* 49(10):653-658 *(In German with English summary)*
NAL Call No. 41.8 T445
Hygienic and therapeutical principles in fish rearing and in production in commercial enterprises, including feeding, breeding, over fishing, sorting, mixing of species, transportation and slaughter are discussed form the animal welfare point. The major points for aquarium fish are related to capture, breeding and trade, as well as from errors in the management and care by aquarists. Game fishing has gained considerable attention with respect to welfare. Examples are presented for all of these diverse activities.
Descriptors: fisheries, fish production, fishing, fish culture, aquaculture, fish farming, animal welfare, fishes

Schnick RA (1996) **Cooperative Fish Therapeutic Funding Initiative: States in partnership with federal agencies to ensure the future of public fish culture.** *Transactions of the North American Wildlife and Natural Resources Conference. pp. 553-55*
NAL Call No. 412.9 N814
The impetus for the Cooperative Fish Therapeutic Funding Initiative was and is the lack of properly approved drugs to reduce disease-related mortality and improve production efficiency and product quality on public aquaculture facilities. This crisis requires more cost-effective methods to gain approval of drugs for use in public aquaculture. Public concerns about human food safety, human health and environmental impacts have resulted in increasingly strict interpretation and enforcement of regulations by the U.S. Food and Drug Administration (FDA). Such actions have drastically curtailed the availability and use of drugs essential to maintain fish health in hatcheries. Drug and chemical manufacturers are reluctant to undertake any significant efforts to gain approval of aquaculture drugs because the market potential for these products is below the potential sales target for research investment (estimated to be $3.5 million for one fish species and one disease). The approval of a drug by FDA can only be obtained with the development of required safety and efficacy

data that leads to a new animal drug application (NADA) that is submitted to FDA for review and approval. The process to generate all the data and have the NADA approved by FDA may take 5 to 10 years. Only three therapeutants and one anesthetic are currently approved and available to hatchery managers. It became apparent to a number of individuals, agencies and organizations that a massive, coordinated and cooperative effort was needed to resolve this crisis. This is the story of how various groups have joined together to meet this awesome responsibility.
Descriptors: drugs, disease control, fish culture, government policy, public health, product development, USA
ASFA; Copyright © 2003, FAO

Schnick RA, Gingerich WH, Koltes KH (1996) **Federal-state aquaculture drug registration partnership: A success story in the making.** *Fisheries. 21(5):4*
NAL Call No. SH1.F54
During the past 20 years, aquaculture has grown both as a vital tool for fisheries management and as a viable industry. But now a crisis has arisen from the Food and Drug Administration's (FDA) increased regulation of drug use in aquaculture in response to public concerns about human food safety, human health, and environmental effects. Lack of approved drugs and chemicals has dramatically reduced the effectiveness and increased the cost of fish production for natural resource management agencies. To make badly needed therapeutants available, the FDA is requiring an array of specialized laboratory research studies and clinical field trials. Pharmaceutical manufacturers are reluctant to undertake any major efforts to gain approval of aquaculture drugs because each (i.e., use on one species for one purpose) is estimated to cost a minimum of $3.5 million. Hence, the expenditure is not warranted by the apparent market potential. Only three therapeutants and one anesthetic are currently approved and available to hatchery managers.
Descriptors: aquaculture products, aquatic drugs, pharmacology, legislation, USA, disease control, aquaculture, pharmaceuticals
ASFA; Copyright © 2003, FAO

Tegge G (1978) **Halterung, Transport und Toten von Fischen. Aus der Sicht des Tierschutzgesetzes vom 24, Juli 1972 [Keeping, transport and killing of fish in relation to Animal Welfare legislation of 24th July 1972].** *Du und das Tier.* 8(1):8-9 (In German)
ISSN: 0341-5759
Descriptors: animal welfare, legislation, transport of animals, fishes

Young JA (1998) **Organic Salmon production and consumption: Ethics, consumer perceptions and regulation.** (Eds:) Barthel KG, Barth H, Bohle-Carbonell M, Fragakis C, Lipiatou E, Martin P, Ollier G, Weydert M. *Third european marine science and technology conference MAST conference, Lisbon, 23 27 May 1998: Project synopses Vol 6: Fisheries and Aquaculture FAIR: 1994-98, selected projects from the research programme for Agriculture and Fisheries including agro industry, food technology, forestry, aquaculture and rural development FAIR. Luxembourg Luxembourg European Commission DG 12 Science, Research and Development.* 6:350-352
The primary project objectives are: 1. Evaluate definitions of organic salmon and aquaculture production from both industry and consumer perspectives. 2. Explore consumers' ethical perceptions of organic salmon in the major EU markets. 3. Critically appraise the technical, animal welfare,and environmental aspects., implicit in organic salmon production in terms of ethical, social, economic and sustainability considerations. 4. Explore critical issues in the

regulatory and legal framework at the national and EU level, thus providing input to regulatory bodies developing standards for organic fish farming at the EU level. These objectives will be achieved through the combined expertise and skills of the academic partners coupled with a key industrial sub contractor involved in the development of organic salmon. This combination will give unique access to data and its analysis though an integrative multi discipline approach. The research programme will provide information to consumers, producers, regulators, environmental and animal welfare groups and others in order to help inform. and determine a framework for consensus and should lirait the potential for negative repercussions to other organic products and conventional salmon production which might result from a poorly regulated move to organic salmon production. Compliance with the work programme: The principal focus for the project addresses many of the aims in the ELSA' programme. In particular the project would seek to assess the. attitudes and perceptions of consumers, producers, animal welfare and environmental, groups and regulatory bodies on the regulation, ethics and other aspects of organic salmon production.
Descriptors: aquaculture, consumers, legislation, organic, salmon

Table 1. National and International Animal Welfare Acts in Relation to Fish

The editor has taken the liberty of researching and providing the reader with selected current national and international animal welfare acts having to do in some part with fish (either specifically including or excluding). This list is not internationally all inclusive, as data from only the selected countries was available. This table of acts is intended to provide the reader with a place to start in searching more about regulations and animal welfare in relation to fish.

Country or Union	Name of Act(s)
Austria	Bundesgesetz vom 27 September 1989 über Versuche an lebenden Tieren (Tierversuchgesetz 1988)
Belgium	Arrêté Royal du 14 novembre 1993 relatif à la protection des animaux d'expérience
European Union	EU-Directive (86/609/EEC)
France	Décret 87-848 du 19 octobre 1987 pris pour l'application de l'article 454 du code pénal et du troisième alinéa de l'article 276 du code rural et relatif aux expériences pratiquées sur les animaux.
Finland	Eläinsuojelulaki 247/1996 (Law on Animal Protection); Eläinsuojeluasetus 396/1996 (Act on Animal Protection); Asetus koe-eläintoiminnasta 1076/1985. (Act on Animal Experimentation, changed partially by act 395/1996); Maa- ja metsätalousministeriön päätös tieteellisten eläinkokeiden luokituksesta 447/1986 (Decree of Veterinary Division in Ministry of Forestry and Agriculture on classification of animal experiments); Asetus kokeellisiin ja muihin tieteellisiin tarkoituksiin käytettävien selkärankaisten eläinten suojelemiseksi tehdyn eurooppalaisen yleissopimuksen voimaansaattamisesta 1360/1990 (Introductory Act on European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes)
Greece	Décret présidentiel du 12-04-1991, FEK A numéro 64 du 03-05-1991 Page 1061
Germany	Erste Gesetz zur Ænderung des Tierschutzgesetzes vom 12-08-1986 Bundesgesetzblatt Teil I vom 22-08-1986 Seite 1309.
Ireland	The Cruelty to Animals Act of 15-08-1876. EC (Amendment of the Cruelty to Animals Acts of 1976) Regulations of 1994, Statutory Instruments Number 17 of 1994.
Italy	DM 27/01/1992 - Attuazione della Direttiva n. 86/609/CEE in materia di protezione degli animali utilizzati a fini sperimentali o ad altri fini scientifici Circolare 05/05/1993 - Decreto Legislativo 27 gennaio 1992, n. 116, articoli 8 e 9, concernenti deroghe agli articoli 3 e 4. Circolare 22/04/1994 n. 8 - Applicazione del Decreto Legislativo 27 gennaio 1992, n. 116, in materia di protezione degli animali utilizzati a fini sperimentali o ad altri fini scientifici.
Norway	The 1974 Animal Welfare Act (in addition supplemented by EU Directive 86/609/CEE)

Table 1. National and International Animal Welfare Acts in Relation to Fish, Continued

COUNTRY OR UNION	NAME OF ACT(S)
Portugal	Decreto-lei numero 129/92 de 15-06-1992., Diario da Republica I Série A, numero 153 de 06-07-1992 Pagina 3197.
Spain	Real Decreto numero 223/88 de 14-103-1988 relativo a la protection de los animales utilizados para experimentacion y otros fines científicos, Boletín Oficial del Estado numero 67 de 18-03-1988 Pagina 8509.
United Kingdom	The Animals (Scientific Procedures) Act 1986 (subsequently amended by three Statutory Instruments)
Sweden	Djurskyddslag', no. 1988/534, amended 25 February 1998 (no. 1998/56)
United States of America	The Animal Welfare Act of 1966 (subsequently amended in 1970, 1976, 1985, 1990, and 2002)

Web Resources:

Animal protection with bioassays – Humane Research: A collection of international laws, regulations, reccomendations.
Tierschutz - Animal Welfare
http://www.uni-giessen.de/tierschutz/

A hint to accessing this site in an English translation: 1) open your web browser. 2) go to http://www.google.com. 3) search http://www.uni-giessen.de/tierschutz/ (the site will be the only site found). 4) next to the title "Tierschutz - Animal Welfare" will be button [translate this page], left click this button. 5) the site will open in an English translation by Google's translation mechanism. (Remember that this is a computer generated translation, so if you need clarification, you will need to go back to the original German site.)

CVM Guide 1240.4200
Low Regulatory Priority Aquaculture Drugs
http://www.fda.gov/cvm/index/aquaculture/LRPDrugs.pdf

CVM Guide 1240.4260
Classification of Aquaculture Species as Food or Nonfood
http://www.fda.gov/cvm/index/policy_proced/4260.pdf

Drugs Approved for Use in Aquaculture
http://www.fda.gov/cvm/index/aquaculture/appendixa6.htm

FDA-CVM guidance document 150
Guidance for Industry, Status of Clove Oil and Eugenol for Anesthesia of Fish
http://www.fda.gov/cvm/index/updates/gl150.htm

Minor Use and Minor Animal Species Health Enhancement Act of 2001
http://www.natlaquaculture.org/MUMASHE%20Act%20White%20Paper%20WEB.htm

The European Union Online
http://www.europa.eu.int/index_en.htm

Thomas: Legislative Information on the Internet
http://thomas.loc.gov/

US Animal Welfare Act and Regulations
http://www.nal.usda.gov/awic/legislat/usdaleg1.htm

US Department of Agriculture, Animal Plant Health Inspection Service, Animal Care
http://www.aphis.usda.gov/ac/

4. THE INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) AND FISH WEB RESOURCES

4.1. Protocol Forms

General Methods For Zebrafish Care
Zebrafish Standard Protocol Form
http://zfin.org/zf_info/zfbook/chapt1/1.9.html

> "The following PDF file contains a template for animal use protocols involving zebrafish which has been approved by the veterinarian (OVSAC) and the animal use committee (IACUC) at the University of Oregon. It is provided to facilitate preparation of applications by users who must meet similar regulations. The text is also available on computer disk."

Animal Use Application to the Institutional Animal Care & Use Committee (IACUC)
University Of Oregon Zebrafish Form
http://zfin.org/zf_info/zfbook/chapt1/anim-zeb.pdf

4.2. Guidelines

99-10: New IACUC Application and USDA Policy 12 Compliance Requirements
http://alpha.ddm.uci.edu/zotmail/archive/1999/19990709109.html

> ... The new IACUC application complies with USDA policy 12. ... 2) Are there any alternative animal models (eg, invertebrates, fish, protozoa, etc.)? ...

AFS Policy Statement #16. American Fisheries Society (AFS), American Society of Ichthyologists and Herpetologists (ASIH), and American Institute of Fishery Research Biologists (AIFRE) (1987) Guidelines for the Use of Fishes in Field Research. Approved Sept 1987, Winston-Salem, NC. Published Mar-Apr 1988, *Fisheries* 13(2):16-23.
http://www.fisheries.org/Public_Affairs/Policy_Statements/ps_16a.shtml
http://199.245.200.110/pubs/fishguide.html

AFS Policy Statement #22. Robinette, H Randall (Chair); Hynes, Julian; Parker, Nick C; Putz, Robert; Stevens, Robert E; and Stickney, Robert R (1990) ABBREVIATED, AFS Policy Statement #22: Commercial Aquaculture. Approved Aug 1990, Pittsburgh, PA. Published Jan-Feb 1991, *Fisheries* 15(2):12
http://www.fisheries.org/Public_Affairs/Policy_Statements/ps_22a.shtml

AFS Policy Statement #30. Kapuscinski, AR and EM Hallerman (1990) Responsible use of Fish and Other Aquatic Organisms. Fisheries 15(4):2-5
http://www.fisheries.org/Public_Affairs/Policy_Statements/ps_30.shtml

> ... Catching and consuming fish are only parts of the value, especially in ... and federally mandated "institutional animal care and use committees" (IACUC), which are ...

Canadian Council on Animal Care guidelines on: the care and use of fish in research, teaching and testing
http://www.ccac.ca/english/new/newframe.htm

> The second draft of the [guidelines] can be downloaded in MS Word format from this site. The final draft will be posted after all input from the second draft is taken into consideration.

DeTolla LJ, Srinivas S, Whitaker BR, Andrews C, Hecker B, Kane AS, Reimschuessel R (1995) **Guidelines for the Care and Use of Fish in Research**. *ILAR Journal*. 37(4)
http://dels.nas.edu/ilar/jour_online.asp?id=jour_online

Fish Care & Information
Arizona State University
http://researchnet.asu.edu/animal_care/animal_information/fish_info.html

IACUC Policies and Procedures Manual
http://researchnet.asu.edu/animal_care/Protocol_Review.pdf
PDF IACUC Policies and Procedures Manual: MS Word | PDF Animal Information Sheets. ...

FSBI (2002) **Fish Welfare.** ***Briefing Paper 2, Fisheries Society of the British Isles, Granta Information Systems***, 82A High Street, Sawston, Cambridge, CB2 4H, Tel/Fax: +44 (0) 1223 830665. Email: FSBI@grantais.demon.co.uk
http://www.le.ac.uk/biology/fsbi/welfare.pdf

IACUC Guideline X: Aquatic Guidelines
Penn State
http://www.research.psu.edu/orp/ani/guide/X.htm
A density of more than 1.5 cm of fish per liter of water should not be ... Each tank must be identified with an identification number assigned by the IACUC. ...

Schwedler TE, Johnson SK (2000) **Responsible Care and Health Maintenance of Fish in Commercial Aquaculture.** ***Animal Welfare Information Center Bulletin, Winter1999/2000.*** Vol. 10, No. 3-4.
http://www.nal.usda.gov/awic/newsletters/v10n3/10n3schw.htm

Thorsteinsson V (2002) **Tagging Methods for Stock Assessment and Research in Fisheries. Report of Concerted Action FAIR CT.96.1394 (CATAG).** Reykjavik. Marine Research Institute Technical Report (79), pp 179
http://www.hafro.is/Timarit/catag.pdf

Westerfield, M. (2000). **The zebrafish book. A guide for the laboratory use of zebrafish (*Danio rerio*). 4th ed.**, Univ. of Oregon Press, Eugene.
http://zfin.org/zf_info/zfbook/zfbk.html

4.3. General Topics

Rose JD (2002) **The Neurobehavioral Nature of Fishes and the Question of Awareness and Pain.** *Reviews in Fisheries Science.* 10(1):1-38
http://uwadmnweb.uwyo.edu/Zoology/faculty/Rose/pain.pdf

Are plants making fish "happy"?
http://zebra.biol.sc.edu/methods/plants.html

A dialog regarding the use of natural plants and an excellent response by Monte Westerfield (Author of THE ZEBRAFISH BOOK: A guide for the laboratory use of zebrafish *Danio* (Brachydanio) rerio*)regarding the potential introduction of pathogens into the system. **...** 25 Mar 1998 12:28 -0400 (EDT) From: hawk0001@mc.duke.edu Subject: Re: (Fwd) Are plants making fish happy ... I would suggest you get help from your IACUC chairperson **...**

AWIC Bulletin: Wildlife Research and the IACUC
http://www.nal.usda.gov/awic/newsletters/v10n1/10n1will.htm

... For fish, our IACUC has determined this same stage of development to be the "buttoned-up" stage, or when the embryo has fully absorbed the yolk sac and must **...**

IACUC Links
University of Alaska - Fairbanks
http://www.uaf.edu/iacuc/links.html

... National Wildlife Health Center, USGS. United States **Fish** and Wildlife Service, USF&WS. National Marine Fisheries Service, NMFS. Other Resources and **IACUC** Sites. **...**

IACUC Newsletter: A look at Fish Welfare (pg. 4)
University of Pittsburg
http://www.iacuc.pitt.edu/Newsletters/Feb2003.pdf

IACUC.org
http://www.iacuc.org/

IACUC Zoonoses
Species Specific Fact Sheets
University of Pennsylvania
http://www.upenn.edu/regulatoryaffairs/animal/zoonoses.html

click on Fish, Reptiles, and Amphibians for a comprehensive table of zoonoses
... Zoonoses (diseases which can be transmitted from animals to humans under natural conditions) by animal species: Birds Dogs and Cats Ferrets Fish, Reptiles, and **...**

Information Regarding the Use of Zebrafish at MBL
Institutional Animal Care & Use Committee
Marine Biological Laboratory, Woods Hole, MA
http://www.mbl.edu/research/services/iacuc/iacuc_zebrafish.html

... Investigators bringing fish from an outside facility to the MBL must choose a method of care for the fish when making an application for IACUC approval. ...

Information Resources for Institutional Animal Care and Use Committees, 1985-1999, revised September 2000. AWIC Resource Series No. 7
http://www.nal.usda.gov/awic/pubs/IACUC/iacuc.htm

Professional Guidelines
http://www.nal.usda.gov/awic/pubs/IACUC/profguid.htm

> ... NAL call number: QL55 A1I43 Descriptors: principal investigator, animal care personnel, IACUC members, overview of current uses of fish in research and commerce ...

Institutional Animal Care and Use Committee (IACUC) Operating Procedure for Release of Research, Instruction, and Display Animals for Adoption
College of Charleston
http://www.orga.cofc.edu/compliance_IACUC_SOP_005.html

National Resource for Zebrafish
Comparative Medicine Resources Directory
National Center for Research Resources, NIH
http://www.ncrr.nih.gov/ncrrprog/cmpdir/FISH.asp#zebra

Northern Arizona University IACUC Self-Instruction Module for Certification in the Humane Care and Use of Laboratory Animals
http://www.for.nau.edu/courses/pb1/FOR690/downloads/IACUC_Training_Module.pdf

> ... State Game and Fish agencies require permits to trap or hold most wildlife species ... Check with the IACUC chair if you have any question regarding permits. ...

Policy on Aseptic Technique
University of Colorado Health Sciences Center
http://www.uchsc.edu/animal/policy%20of%20aseptic%20technique.htm

> ... For example, skin preparation in amphibians and fish is not recommended as such ... component of the research protocol and approved by the IACUC, non-rodent ...

Veterinarians In Research Labs Address Conflicting Agendas
http://www.the-scientist.com/yr1997/may/finn_p1_970526.html

> ... I hope they were humane when they killed the fish.". ... of the Washington, DC-based Humane Society of the United States, for example, think IACUC veterinarians are ...

Zebrafish at UCL
http://www.ucl.ac.uk/zebrafish-group/

IACUC Links
http://www.ucl.ac.uk/zebrafish-group/links/links.html

4.4. Anesthesia and Analgesia

Small Animal Survival Surgery Guide
Northeastern Ohio Universities College of Medicine, Division of Basic Medical Sciences
http://www.neoucom.edu/DEPTS/CMU/PIhandbook/SmallAnimalSurvivalSurgery.html

> ... applicable to survival surgery in small amphibians, reptiles, and fish with appropriate modifications. Other species may be added as needed with IACUC approval ...

4.5. Euthanasia and Endpoints

CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing
Acute Toxicity Testing in Fish
http://www.ccac.ca/english/gui_pol/gdlines/endpts/APP9TO10.HTM

> ... justified for reasons of scientific necessity and approved by the institutional animal care and use committee (IACUC). ... Acute toxicity studies in fish. ...

Euthanasia Guidelines
University of Minnesota
http://www.ahc.umn.edu/rar/euthanasia.html

Euthanasia Protocol
SOP-160 Euthanasia
http://www.bgsu.edu/offices/spar/IACUC/IACUC%20web%20documents/SOP-160%20Euthanasia.doc

> ... procedure with explicit IACUC approval. Cooling and/or Freezing. Liquid nitrogen. Total immersion of prenatal rats and mice or larval forms of fish in liquid ...

Guidelines for Euthanasia
University of California, Riverside
http://www.ora.ucr.edu/vet/Primer/Biomethodology/Euthanasia.htm

Table of Euthanasia Methods
http://www.ora.ucr.edu/vet/Primer/biomethodology/euthanasia.htm#FISH

IACUC Guideline XI
Electrofishing Guidelines
Penn State
http://www.research.psu.edu/orp/ani/guide/XI.htm

Methods of Euthanasia by Species
Euthanasia Guidelines
University of Michigan
http://advs.usu.edu/LARC/euthgd.htm

Policy for Euthanasia of Research Animals
UCI Animal Subjects
http://www.rgs.uci.edu/as/vieuth.htm

... Fish, Barbiturates Inhalant anesthetics CO2, Tricaine methane sulfonate (TMS, MS222 ... 2. All euthanasia methods must be described in an approved IACUC protocol. ...

UW Policy for the Euthanasia of Fish Species - 2002
http://depts.washington.edu/compmed/iacuc/policies/fish_euthanasia.html

... UW Policy for the Euthanasia of Fish Species - 2002. Approved July 18, 2002. ... A. Primary euthanasia methods for fish (methods that can be used alone) include: ...

4.6. Laws, Regulations, and Governing Entities

IACUC Learning Module: Laws and Regulations
Laws, Guidelines & Programs Affecting Animal Research
The University of Arizona
http://www.iacuc.arizona.edu/training/laws/research.html

IACUC Learning Module - Laws & Regulations... Arizona Game & Fish And Agencies Such As Fish & Wildlife Service: ...

Regulations, Guidelines, and Miscellaneous Recommendations
University of Idaho
http://www.uro.uidaho.edu/committees/acuc/resources/regulations.htm

... Fish & Wildlife, ... IACUCs, Institutional Animal Care and Use Committee Guidebook. Wildlife Research and the IACUC - Animal Welfare Information Center. ...

Regulations and Governing Bodies
Office of the University Veterinarian
University of Kentucky Research
http://www.mc.uky.edu/dlar/resources/handbook/regulations.htm

Federal Agencies, Laws, Regulations and Policies
The Freedom of Information Act
Independent National Regulatory Bodies and Professional Groups

5. EDUCATIONAL TRAINING MATERIALS & COURSES

Care, Handling and Use of Aquatic Animals
The Canadian Aquaculture Institute, a division of AVC Inc.
http://www.upei.ca/fishcarecourse/

"Fish and other aquatic animals have unique characteristics and needs, especially in closed environments. In recent years, fish have become increasingly important in research, teaching and testing. In the Canadian Council on Animal Care's (CCAC) Animal Use Survey (1999), fish accounted for approximately 23% of all research animals in Canada. .... personnel who carry out animal-based work will receive adequate training in the principles of laboratory animal science and the ethical issues involved in animal use. The Care, Handling and Use of Aquatic Animals program has been specifically tailored to meet NIAUT guidelines and to address the specific care and use issues associated with aquatic animals in laboratory settings."

"The Care, Handling and Use of Aquatic Animals program is an intensive, broad-based program that is an ideal introductory or refresher course for individuals involved in aquatic animal research, teaching or testing."

Topics: Regulation of Experimental Animal Use in Canada; Ethical Issues of Experimental Animal Use; The Three Rs (Replacement, Reduction, Refinement); Occupational Health and Safety

Fish as a Research Animal; Recognizing Pain, Distress and Stress in Fish; Aquatic Animal Biology and Husbandry Considerations; Anesthesia and Euthanasia; Routine Techniques Using Aquatic Animals (Weighing and Measuring, Body Fluid Sampling, Tagging and Marking); Water Quality and Laboratory Holding Systems; Health and Disease of Aquatic Animals; Post Mortem Techniques

The Experimental Fish: Institutional Aquatic Animal User Training
The Canadian Aquaculture Institute Online
http://www.upei.ca/cai/experimentalfish.htm

"The Experimental Fish is an online WebCT training program for people who "use" fish in research, teaching and testing applications. The course syllabus closely follows the core topics outlined in the Canadian Council on Animal Care's *National Institutional Aquatic Animal User Training Program* with special reference to aquatic animals. This program reviews not only regulatory and ethical issues involved in experimental animal use but also the practical aspects of aquatic animal care. For institutions that are provide training programs for animal user staff, this course in combination with wet-lab demonstrations will provide a complete user training program."

"Coming Soon - U.S. Version of *The Experimental Fish*

This version will include modules on American animal welfare legislation and regulatory systems, IACUC roles and responsibilities, and other issues of specific concern pertaining to fish research in the United States. This program is planned for release in the Spring of 2003."

Fish Anesthetization and Recovery, Marking and Tagging Techniques, and Body Fluid Sampling. (CD–ROM)
The Canadian Aquaculture Institute, a division of AVC Inc.
http://www.upei.ca/cai/experimentalfish.htm

"CAI is currently developing aquatic animal training media (e.g. CD-Rom format) on a variety of issues including *Fish Anesthetization and Recovery*, *Marking and Tagging Techniques*, and *Body Fluid Sampling*. These additional training tools are expected to be available for purchase from CAI later in 2003."

The Canadian Aquaculture Institute
Continuing Education and Training in Aquaculture and Fish Health
Publications & Videos
http://www.upei.ca/~cai/publications.html

"The Canadian Aquaculture Institute has developed both manuals and videos on a variety of topics including recirculation systems, fish health, broodstock technologies and fish bath treatments."

Fish Welfare Officer Training Course (Quality & Welfare of Farmed Salmon & Trout)
AWTraining
http://www.awtraining.com/tran/tranfs/fwo.html

"This two day course will explain in detail those aspects of commercial fish production that directly affect welfare and subsequent carcass and meat quality. It is at present aimed at farmed salmon and trout, and relates to the physiological, biochemical, moral, ethical and legislative components of production, harvesting, transportation, off-loading and processing drawing on the latest research in this subject area. The first FWO Course is scheduled to launch on 29th / 30th April 2003. Aspects which are particularly attractive to the industry are the emphasis given to the links between 'best practice' and flesh quality as well as the information on new and impending legislation. The course explains the detailed background science behind commercial production including veterinary, research, commercial and consumer perspectives, legislative requirements and practical detail accommodating the international nature of this rapidly growing industry. Video material illustrating good practice is incorporated using a customised computer-controlled, multimedia presentation package. Discussion groups form an important part of the course allowing delegates to apply the principles to their individual situations. Successful completion results in an AWTraining certificate of attendance."

Norwegian Aquamedicine Education and Training
http://www.veso.no/aquamedicine/education.html

6. COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE (CSREES) REPORTS CURRENT RESEARCH INFORMATION SYSTEM REPORTS (CRIS)

ACCESSION NO: 0188571 **SUBFILE:** CRIS
PROJ NO: WVAX-BIOPLEX **AGENCY:** CSREES WVAX
PROJ TYPE: OTHER GRANTS **PROJ STATUS:** EXTENDED
CONTRACT/GRANT/AGREEMENT NO: 2001-38850-10529 **PROPOSAL NO:** 2002-036203
START: 15 JUN 2001 **TERM:** 14 JUN 2003 **GRANT YR:** 2002
GRANT AMT: $561,600

INVESTIGATOR: Chatfield, J. M.; Liedl, B.; Huber, D.; Ruhnke, T.

PERFORMING INSTITUTION:
BIOLOGY
WEST VIRGINIA STATE COLLEGE
PO BOX 1000
INSTITUTE, WEST VIRGINIA 25112

ORGANIC WASTE TREATMENT USING THERMOPHILIC ANAEROBIC DIGESTION (BIOPLEX) PHASE 2

OBJECTIVES: 1) Develop a fermentation laboratory containing continuous stir tank, anaerobic filter and fluidized bed anaerobic bioreactors. Establish physical and biochemical parameters to maintain and transfer these discoveries to the industrial sector. 2) The reduction in pathogens during digestion will be studied, with emphasis on *Cryptosporidium*, *Salmonella* and *E. coli*. 3) Determine the feasibility of using microbial protein from anaerobically digested poultry litter as a substitute for fishmeal in trout feeds will be determined. 4) Evaluate the microbial community dynamics of thermophilic anaerobic digesters. 5) Demonstrate the efficacy of digested poultry litter solids as a replacement for commercial fertilizers. As well as, evaluate a hydroponic system for capacity to remediate liquid effluent.

APPROACH: Fifty-liter laboratory digesters and a 10,000-gallon pilot plant digester will be used to refine the biology, biochemistry and control of anaerobic digestion. Promising discoveries will be demonstrated in the pilot plant thus establishing immediate industrial application. Fish feedstock and fertilizer experiments using both solid and liquid degester effluents are planned to establish novel applications and potential commercial value.

NON-TECHNICAL SUMMARY: Increasing production of agricultural waste associated with farming activities impacts health, economic and environmental welfare. The "Bioplex" project is comprised of five research projects involving the utilization of agricultural waste and thermophilic anaerobic digestion. Innovations and developments resulting from these studies will result in both more efficient and commerically viable digesters.

PROGRESS: 2001/01 TO 2001/12
1. Construction of the fermentation lab was completed and 3 digester model types were seeded to establish microbial communities. 2. A 20-day *Cryptosporidium muris* oocyst removal study was performed. Results showed a 97% removal of oocysts. Experiments using *Cryptospordium parvum* have commensed. 3. Aquaculture experiments have be delayed due to troubleshooting of the water cooling system in the aquaculture lab. Feed trials using recovered microbial proteins from the pilot plant digester will begin in June. 4. Characterization of the microbial community in the pilot plant

reactor is underway. Using Bacteria and Archea specific primers, we have PCR-amplified 16S rDNA from extracted total community DNA. These were used for construction of two 16S rRNA gene libraries using TA cloning. 5. Raised bed fertilizer trials consisted of 4 treatments and a control on blueberries, tomatoes, potatoes and corn. Results showed clear responses to fertilzation using the digested litter.

PUBLICATIONS: 2001/01 TO 2001/12
Stafford, DA; Ruhnke, T, Huber, D; Chatfield, M and Hubbard, H. 2002. Microbial Diversity of Thermophilic Anaerobic Digesters and Control in *Cryptosporidium muris* Oocyst Removal. World Water Congress Proceedings (April 2002 Melbourne, Australia).

PROJECT CONTACT:

Name: Morris, K. R.
Phone: 304-766-4165
Fax: 304-766-4199
Email: morriska@mail.wvsc.edu
URL: http://www.wvsc.edu/~chatfima

ACCESSION NO: 7001809 **SUBFILE:** ICAR
PROJ NO: 16970 **AGENCY:** UNIVERSITIES **TERM:** 30 APR 1998 **FY:** 1997

INVESTIGATOR: MOCCIA R; TECH

PERFORMING INSTITUTION:
UNIVERSITY OF GUELPH ONTARIO AGRICULTURAL COLLEGE
Guelph, Ontario N1G 2W1

DEVELOPMENT OF QUANTITATIVE PHYSIOLOGICAL MEASURES OF THE WELFARE STATUS OF CULTURED SALMONID FISHES.

NOTES: CONTACT: MOCCIA R; PHONE: 519-824-4120

ACCESSION NO: 7001647 **SUBFILE:** ICAR **FY:** 1997

INVESTIGATOR: GRANT J W A; ROBB S; STEINGRIMSSON S; TECH

PERFORMING INSTITUTION:
CONCORDIA UNIVERSITY
1455 de Maisonneuve Boulevard West
Montreal, Quebec H3G 1M8

RESOURCE DEFENSE BEHAVIOUR IN FISHES

NARRATIVE: IMPACT: Our research has implications for aquaculturists who want to maximize production of uniformly sized fish. This can be accomplished by presenting food in such a way to reduce aggression and monopolization of food within groups. We have developed a simple regression model to predict stocking densities of salmonid fish in the wild and in aquaculture conditions.

OBJECTIVES: To determine how resource distribution (food and mates) influences the aggressive behaviour and uneven distribution of resources within a group of fishes. To determine whether territory size limits the density of stream-dwelling salmonid fishes.

PROGRESS:
Aggressive behaviour and monopolization of resources increase when resources are spatially clumped, temporally dispersed and spatially predictable. Territory size is a good predictor of the maximum density of juvenile salmonids in streams and of the occurrence of density-dependent population regulation.

NOTES: CONTACT: GRANT J; PHONE: 514-848-3421; FAX: 514-848-2881; COOPERATING ESTABLISHMENT: FISHERIES AND OCEANS CANADA, GULF REGION, SCIENCE BRANCH, P.O. Box 5030, Moncton, New Brunswick, E1C 9B6 (C104005); COOPERATING ESTABLISHMENT: MCGILL UNIVERSITY, 845 Sherbrooke Street West, Montreal, Quebec, H3A 2T5 (C305012); COOPERATING ESTABLISHMENT: UNIVERSITY OF GUELPH, COLLEGE OF BIOLOGICAL SCIENCE, Guelph, Ontario, N1G 2W1 (C306008); FUNDING ESTABLISHMENT: NATURAL SCIENCES AND ENGINEERING RESEARCH COUNCIL, Constitution Square, 350 Albert Street, Ottawa, Ontario, K1A 1H5 (F111053)

ACCESSION NO: 7000413 **SUBFILE:** ICAR
PROJ NO: 2 **AGENCY:** OTHER FEDERAL
START: 01 APR 1990 **TERM:** 31 MAR 1994 **FY:** 1997

INVESTIGATOR: DE MARCH B G E

PERFORMING INSTITUTION:
FISHERIES AND OCEANS CANADA, CENTRAL AND ARCTIC REGION FRESHWATER INSTITUTE
501 University Crescent
Winnipeg, Manitoba R3T 2N6

INCUBATION METHODOLOGY IN ARCTIC CHARR

NARRATIVE: IMPACT: Egg and juvenile supply is a major factor limiting the development of the Arctic charr aquaculture industry. Methods of improving egg quality had to be identified. In fact, very low temperatures may be required for successful incubation.

OBJECTIVES: This experiment was done with the objective of finding methods of improving hatching success.

PROGRESS:
Project completed 1995. Manuscript "Effects of incubation temperature on the hatching success of Arctic charr (*Salvelinus alpinus*)" has been submitted for publication in Prog. Fish-Cult.

NOTES: CONTACT: DE MARCH B G E; PHONE: 204-983-5213; FAX: 204-984-2403; COOPERATING ESTABLISHMENT: FISHERIES AND OCEANS CANADA, ROCKWOOD AQUACULTURE RESEARCH CENTRE, Gunton, Manitoba, R0C 1H0

7. RECORDS FROM THE NATIONAL AGRICULTURAL LIBRARY ELECTRONIC CATALOG

NAL CALL NO: 389.8 V26 No.7-8
Author: Westoo, Gunnel.
Title: Metylkvicksilverhalter i fisk fangad mars 1968-april 1971[Methylmercury levels in fish caught March 1968-April 1971.] Socialsytrelsens och veterinarstyrelsens rekommendationer betraffande fiskkonsumtion. [Recommendations as to fish consumption by the National Board of Health and Welfare and the National Veterinary Board. Report on mercury in foods by the joint FAO/WHO Expert Committee on Food Additives], 1970
Publisher: Stockholm
Description: 179-321 p. illus., map
Series: Var foda, no. 7-8

NAL CALL NO: SF457.3 H69 1991
Title: How to choose an aquarium for your pet fish.
Publisher: Potters Bar [England] : Universities Federation for Animal Welfare, [1991]
Description: 1 folded sheet (4 p.) : ill. ; 21 cm.
Note: Cover title.
Note: "April 1991"--P. [4].
LC Subject: Aquarium fishes -- Housing.
LC Subject: Fishes -- Housing.
LC Subject: Aquariums.
Other Author: Universities Federation for Animal Welfare.

NAL CALL NO: aZ7994 L3B47 1992
Author: Jensen, D'Anna J. B.
Title: Information resources for reptiles, amphibians, fish, and cephalopods used in biomedical research / D'Anna J. Berry, Michael D. Kreger, Jennifer L. Lyons-Carter.
Publisher: Beltsville, Md. : U.S. Dept. of Agriculture, National Agricultural Library, Animal Welfare Information Center, [1992]
Description: iii, 87 p. : ill. ; 28 cm.
Note: "December 1992"--Cover.
Note: Includes index.
LC Subject: Animal models in research -- Bibliography.
LC Subject: Reptiles as laboratory animals -- Bibliography.

NAL CALL NO: SF406 C35 1992
Title: The Care and use of amphibians, reptiles, and fish in research / edited by Dorcas O. Schaeffer, Kevin M. Kleinow, and Lee Krulisch ; [illustrations by Barbara Bonner].
Publisher: Bethesda, Md. (4805 St. Elmo Ave., Bethesda 20814) : Scientists Center for Animal Welfare, [1992]
Description: vii, 196 p. : ill. ; 28 cm.
Note: Includes bibliographical references.
Note: "Proceedings from a SCAW/LSUSVM-sponsored conference ...held April 8-9, 1991 in New Orleans, Louisiana ..."
Note: "November 1992."
Note: Regulations and guidelines -- Regulations and guidelines:

NAL CALL NO: QL55 A12 B4 1995
Gov Doc No.: A 17.18/2:IN 3/995
Author: Jensen, D'Anna J. B.
Title: Information resources for reptiles, amphibians, fish, and cephalopods used in biomedical research / D'Anna J.B. Jensen, Michael D. Kreger, Jennifer L. Lyons-Carter.
Edition: [Rev. ed.].
Publisher: Beltsville, Md. (10301 Baltimore Blvd., Beltsville 20705) : U.S. Dept. of Agriculture, National Agricultural Library, Animal Welfare Information Center, 1995.
Description: iii, 90 p. : ill. ; 28 cm.
Note: Shipping list no.: 95-0274-P.
http://www.nal.usda.gov/awic/pubs/oldbib/reptiles.htm

NAL CALL NO: SF105 W67 1998
Author: World Congress on Genetics Applied to Livestock Production (6th : 1998 : University of New England)
Title: Proceedings of the 6th World Congress on Genetics Applied to Livestock Production : Armidale, NSW, Australia, January 11-16, 1998.
Other Title: Proceedings of the Sixth World Congress on Genetics Applied to Livestock Production
Publisher: Armidale, NSW : University of New England, 1998.
Description: 6 v. : ill. ; 25 cm. + 1 computer optical disc (4 3/4 in.) + 1 program (iv, 46 p. : ill. ; 25 cm.)
Note: Includes bibliographical references and index.

NAL CALL NO: aHV4701 A94 no. 10
Author: Crawford, Richard L.
Title: Information resources for amphibians, fish & reptiles used in biomedical research / Richard L. Crawford, D'Anna Jensen, Tim Allen.
Publisher: Beltsville, MD : U.S. Dept. of Agriculture, National Agricultural Library, Animal Welfare Information Center, [2001]
Description: vi, 111 p. ; 28 cm.
Series: AWIC resource series, no. 10
Access Info.: http://www.nal.usda.gov/awic/pubs/amphib.htm
Note: “August 2001”--Cover.

APPENDIX 1. Aquaculture & Fisheries Professional Associations, Groups, and Societies

American Fisheries Society
http://www.fisheries.org

American Institute of Fishery Research Biologists (AIFRB)
http://www.iattc.org/aifrb

American Society of Ichthyologists and Herpetologists
http://199.245.200.110

Aquaculture Association of Canada (Association Aquacole du Canada)
http://www.aquacultureassociation.ca

Aquacultural Engineering Society
http://www.aesweb.org

Aquatic Ecosystem Health & Management Society
http://www.aehms.org

Asian Fisheries Society
http://www.compass.com.ph/~afs

Australian Society of Fish Biology
http://www.asfb.org.au

Challenger Society for Marine Science
http://www.soc.soton.ac.uk/OTHERS/CSMS

China Society of Fisheries
http://www.fisheries.moa.gov.cn

Estuarine and Coastal Sciences Association
http://www.ecsa.ac.uk

European Aquaculture Society
http://www.easonline.org

Fisheries Society of the British Isles
http://www.le.ac.uk/biology/fsbi

Global Aquaculture Alliance
http://www.gaalliance.org/

International Association of Aquaculture Economics and Management
http://www.uq.edu.au/aem/index.html

Israel Society for Oceanography and Marine Sciences
http://marine.ocean.org.il

Malaysian Fisheries Society
http://malaysianfisherysociety.virtualave.net

National Aquaculture Association
http://www.natlaquaculture.org

National Shellfisheries Association
http://www.shellfish.org

New York State Aquaculture Association
http://www.morrisville.edu/nysaa

Scottish Association for Marine Science
http://www.sams.ac.uk

Sociedade Brasileira de Ictiológia
http://www.sbi.bio.br

Somali Fisheries Society
http://www.soma-fish.net

Texas Aquaculture Association
http://www.texasaquaculture.org

Western Europe Fish Technologists Association
http://www.wefta.nl

World Aquaculture Society
http://www.was.org

World Conservation Union
http://www.iucn.org

APPENDIX 2.
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